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# INCOMPATIBILITY IN *COSMOS BIPINNATUS*

LESLIE K. CROWE
*John Innes Horticultural Institution, Hertford*

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## I. INTRODUCTION

THE majority of self-incompatible plants in which the genetic basis of the mating system is known, belong to one of two classes. First there are the heteromorphic plants in which the genetic mechanism for outcrossing consists of one or two genes each with two alleles. There is dominance between the alleles in the pollen and in the style. The behaviour of the pollen is determined by the genotype of the mother plant, in other words by the sporophyte. *Primula* species are typical of this group. Secondly there are homomorphic plants in which there is one incompatibility gene with numerous alleles each acting individually in the pollen and in the style. The pollen behaviour is determined by the gametophyte. *Nicotiana*, *Œnothera* and *Trifolium* species are typical of this class.

Recent workers on *Parthenium argentatum* (Gerstel, 1950) and *Crepis fœtida* (Hughes and Babcock, 1950) have described a novel type of incompatibility. To explain the results, one incompatibility gene is postulated with multiple alleles which act individually in the style as in *Nicotiana*, but the behaviour of the pollen is determined by the sporophyte and the alleles exhibit dominance, both features associated with the heteromorphic outbreeeding types. Thus, for the first time a system has been described in two species of the Compositæ which combines genetic features typical of both the heteromorphic and homomorphic types of incompatibility. Regular features of this new system are reciprocal differences in compatibility, incompatibility between some $F_1$ plants and one or both parents, and plants which are homozygous for one allele.

There are two examples of this new breeding system in the Compositæ and it is therefore of interest to find out whether it is typical of the family as a whole. A report on incompatibility in *Cosmos bipinnatus* (Little, Kantor and Robinson, 1940) concluded that the mating system was similar in all respects to that of *Nicotiana*. The experiments, however, were not designed to show reciprocal differences in compatibility, or incompatibility between progeny and parents and these must be considered if the Composite type of system is to be distinguished from any other. This paper describes a reinvestigation of the mating behaviour of *C. bipinnatus*.

## 2. MATERIALS AND METHODS

Seed from open pollinated flowers of *C. bipinnatus* ($2n = 24$) was obtained from the Chelsea Physic Garden. Since this species is an annual, special measures were necessary to permit crossing between parents and their progeny. Firstly, by sowing seed as soon as it was ripe, the time lag due to dormancy was avoided. (Flowering started about twelve weeks after germination irrespective of the season.*) Secondly, parent plants were cut back regularly which prolonged their life until the progeny were in flower.

Pollinations for seed were made by dusting two heads together. Repeated self-pollinations on 12 plants failed to produce a single seed and consequently throughout the course of the work flowers were not emasculated. Compatible pollinations produce 20-30 seeds per head.

Compatibility was later determined solely by examination of pollen tube growth in the style. After pollination the heads were cut and transferred to an incubator running at 27° C. The styles were removed from the flowers 48 hrs. later, fixed in 1 : 3 acetic alcohol and stained in acid fuchsin and light green (Darlington and La Cour, 1947). Satisfactory staining takes 12-15 hrs. at 25° C. After an incompatible pollination the pollen grains germinate but the tubes seldom penetrate beyond the first layer of cells in the stigma and many are washed off in the process of fixing and staining. Compatible ones grow down well into the style.

The progeny from five crosses made on the original plants were studied in detail. Five parental plants were involved and these will be represented by the symbols PI, PII, PIII, PIV and PV. The crosses studied were (1) PI × PII, (2) PIII × PIV, (3) PIV × PIII, (4) PIV × PV and (5) PV × PIV. The results from the five crosses will be described in order, starting with PI × PII.

## 3. RESULTS

Twelve plants of the progeny from the cross PI × PII were selfed and reciprocally intercrossed in all possible combinations. There were four mating groups which will be referred to as GI, GII, GIII and GIV. The proportion of plants in each group was 5, 2, 3 and 2 respectively.

The plants within a group are all cross incompatible. As females, plants in GI are compatible with GIV only, while as pollen parents, GI plants are compatible on GIII and GIV stigmas but not with GII. There is therefore a reciprocal difference in crosses involving plants in GI and GIII. Group GII is reciprocally compatible with GIII and GIV which are themselves reciprocally compatible. The results are summarised in table 1.

All plants were reciprocally crossed with the two parents PI and PII. The results are given in table 2.

Plants in classes GI and GII are reciprocally incompatible with the female parent. With the male parent the same plants are compatible with the exception of the cross with GI as female, which is incompatible. Classes GIII and GIV are reciprocally compatible with the female parent but GIV only is compatible with the male.

* *C. bipinnatus* is always classified as a "short day" plant. It is probable that, in captivity, selection has favoured plants with less rigid light requirements. Many horticultural varieties are now indifferent to day length in respect of flowering time. Recently a family of *Cosmos* segregated into plants which flowered in "long day" conditions and others which did not. The genetics of this difference is being investigated.

These results cannot be explained on the *Nicotiana* scheme proposed by East for, on this system, if the progeny from a cross fall into four groups, the groups are always cross compatible. In *Cosmos*, (i) the incompatibility between different mating types in the $F_1$, (ii) incompatibility between parent plants and some of the offspring, and (iii) reciprocal differences in incompatibility are all features foreign

TABLE 1

*Incompatibility relations of 12 plants of the progeny from the cross* P I × P II

| ♀ \ ♂ | G I | G II | G III | G IV |
|---|---|---|---|---|
| G I | − | − | − | + |
| G II | − | − | + | + |
| G III | + | + | − | + |
| G IV | + | + | + | − |

− = incompatible. + = compatible.

to the *Nicotiana* system in which they occur only rarely and then as a result of illegitimate matings induced by special techniques such as bud pollination.

Turning to the Composite scheme proposed by Gerstel, Hughes and Babcock, again we find that although many of the results can be explained on this scheme it is not sufficient to account for all the data. By modifying this scheme, however, we can explain the facts.

TABLE 2

*Results of back-crossing the progeny of* P I × P II *with the parents (reciprocally)*

| $F_1$ groups | P I × $F_1$ | Reciprocal | P II × $F_1$ | Reciprocal |
|---|---|---|---|---|
| G I | − | − | + | − |
| G II | − | − | + | + |
| G III | + | + | − | − |
| G IV | + | + | + | + |

Let us follow Gerstel and postulate **R** as the incompatibility gene and the subscripts 3, 4, 5 and 6 as the alleles. (Alleles are allocated numbers which reflect their dominance relations, *e.g.* **R**1 is dominant to **R**2 and both are dominant to **R**3.) Then the original cross can be represented **R**3,5 × **R**4,6 and the four classes in the progeny will be **R**3,4, **R**3,6, **R**4,5 and **R**5,6 respectively.

From the results we can deduce that the behaviour of the pollen of the first three types is determined sporophytically and that **R**3

is dominant to the other three alleles and **R4** is dominant to **R5** and **R6**. To account for the behaviour of **R5,6** genotypes, a deviation from the scheme is necessary since the results can only be explained if the alleles **R5** and **R6** are under gametophytic control. In this respect, the system in *Cosmos* that we have suggested differs from *Parthenium* and *Crepis* in which pollen is always under sporophytic control.

It is possible to modify the Composite system in another way which would explain the facts equally well. If we assume that some alleles are dominant in the style as well as in the pollen and we represent the cross PI × PII as **R3,5** × **R4,5** then the four groups in the progeny would be **R3,4**, **R3,5**, **R4,5** and **R5,5** respectively. In this

TABLE 3

*A comparison of the results of crossing* GIV *with* PI, PII, GII *and* GIII *based on the two schemes discussed in the text*

| Cross | Gametophytic and sporophytic pollen determination | | Dominance and individual action of alleles in the style | |
|---|---|---|---|---|
| | Genotype | Progeny expected | Genotype | Progeny expected |
| PI × GIV | **R3,5** × **R5,6** | **R3,6** ; **R5,6** | **R3,5** × **R5,5** | **R3,5** ; **R5,5** |
| Reciprocal | **R5,6** × **R3,5** | **R3,5** ; **R3,6** ; **R5,5** ; **R5,6** | **R5,5** × **R3,5** | **R3,5** ; **R5,5** |
| PII × GIV | **R4,6** × **R5,6** | **R4,5** ; **R5,6** | **R4,5** × **R5,5** | **R4,5** ; **R5,5** |
| Reciprocal | **R5,6** × **R4,6** | **R4,5** ; **R4,6** ; **R5,6** ; **R6,6** | **R5,5** × **R4,5** | **R4,5** ; **R5,5** |
| GII × GIV | **R3,6** × **R5,6** | **R3,5** ; **R5,6** | **R3,5** × **R5,5** | **R3,5** ; **R5,5** |
| Reciprocal | **R5,6** × **R3,6** | **R3,5** ; **R3,6** ; **R5,6** ; **R6,6** | **R5,5** × **R3,5** | **R3,5** ; **R5,5** |
| GIII × GIV | **R4,5** × **R5,6** | **R4,6** ; **R5,6** | **R4,5** × **R5,5** | **R4,5** ; **R5,5** |
| Reciprocal | **R5,6** × **R4,5** | **R4,5** ; **R4,6** ; **R5,5** ; **R5,6** | **R5,5** × **R4,5** | **R4,5** ; **R5,5** |

When alleles are dominant they are underlined

case the behaviour of the three alleles in the pollen must be sporophytically determined, **R3** being dominant to **R4** and **R5**, and **R4** dominant to **R5**. In the style **R3** and **R4** must be assumed to act individually when they occur together but both are dominant in combination with **R5**.*

The results expected on the alternative theories accord completely with those obtained.

It is possible to decide which of the two theories proposed is correct by reciprocally crossing plants of GIV with the parents and with plants of GII and GIII since the results expected from these crosses are different on the two theories. Considering the first theory, GIV

* The evidence can also be interpreted on the basis that the parents are **R3,5** and **R4,6** and the pattern of dominance in the style is $\frac{\mathbf{R3}}{\mathbf{R4}} \rightarrow \frac{\mathbf{R5}}{\mathbf{R6}}$ and in the pollen is $\mathbf{R3} \rightarrow \mathbf{R4} \rightarrow \frac{\mathbf{R5}}{\mathbf{R6}}$. In the interests of brevity and clarity, only the simpler explanation involving 3 alleles is discussed in the text, since the principles involved are the same in both cases.

plants will be **R**5,6 and when they are crossed as females will give rise to progeny consisting of four mating groups, but as males only two groups will occur in the progeny. On the other hand, following the second theory when GIV plants will be **R**5,5, the same reciprocal crosses will produce only two mating groups in the progeny whichever way they are made, and furthermore the groups will be the same in the progeny from reciprocal crosses. Table 3 compares the results expected from these crosses in the case of each theory. It was not possible to make crosses between the female parent, PI, and plants of GIV because the PI plant had stopped flowering, but all the other crosses were made and the progeny tested by intercrossing sibs in all possible combinations. The results are given in Table 4.

TABLE 4

*An analysis of the progeny from* 6 *test crosses between* GIV *and the* 3 *plants* PII, GII *and* GIII

| | PII | R4,5 | GIII | R4,5 | GII | R3,5 |
|---|---|---|---|---|---|---|
| GIV ♀ R5,5 | GV | 7 | GVI | 6 | GXIV | 5 |
| | GIX | 6 | GX | 5 | GXI | 4 |
| GIV ♂ | GVII | 7 | GVIII | 7 | Slightly self-compatible | |
| | GXII | 6 | GXIII | 6 | | |

The numbers opposite the groups represent the number of plants in the group.

The progeny from each cross can be divided into two clear cut intra-sterile inter-fertile groups with one exception. The progeny from the cross GII × GIV were slightly self-compatible, producing 2 or 3 seeds per head after self-pollination. The reason for this is not certain but these plants had been in flower for some time before any pollinations were made on them and it is possible that the results were due to pseudo-fertility, reported by many workers to occur in self-incompatible plants towards the end of the season.

The results obtained are those anticipated on the theory that there is both individual action and dominance of alleles in the style depending on the combination of alleles present. They rule out the possibility of gametophytic pollen determination.

The two right hand columns in table 3 show that if the proposed scheme is correct, the genotype **R**5,5 should occur in the progeny of each cross and **R**4,5 in the progeny from the reciprocal crosses between GIV and PII and GIV and GIII. **R**3,5 should occur in the progeny from crosses between GII and GIV. We already know the behaviour of these genotypes when they are intercrossed, therefore by selecting one plant from each of the two groups in the progeny of all six test crosses and intercrossing them we should get additional evidence in support of the proposed scheme. This was done: the self-fertile

plants from the cross GII × GIV being omitted. A plant in class GI (**R**3,4) was included in these crosses which should therefore include all four genotypes occurring in the progeny of the original cross PI × PII. The results are recorded in table 5, and it will be seen that, as expected, the mating reactions form exactly the same pattern as that recorded in table 1.

Plants classified on the results as **R**5,5 occur in the progeny of every test cross as expected and **R**3,5 and **R**4,5 types also occur in the progeny as forecast. Thus we have proof that the deviation from the composite type of mating system found in *Cosmos* amongst the

TABLE 5

*The inter-relations of progeny obtained by crossing PII, GII, and GIII with GIV*

| ♀ \ ♂ | GI | GXIV | GV-GVIII | GIX-GXIII | |
|---|---|---|---|---|---|
| GI | − | − | − | + | **R**3,4 |
| GXIV | − | − | + | + | **R**3,5 |
| GV-GVIII | + | + | − | + | **R**4,5 |
| GIX-GXIII | + | + | + | − | **R**5,5 |
| | **R**3,4 | **R**3,5 | **R**4,5 | **R**5,5 | ♂ \ ♀ |

progeny of the cross PI × PII can be attributed to dominance of alleles in the style.

* * * * * *

Further evidence of dominance of alleles in the style was obtained from the progeny from reciprocal crosses between PIII and PIV. Amongst 19 progeny from the cross PIII × PIV, 247 out of the possible 361 different pollinations were made with the result that 2 mating groups, GXV and GXVI were found in the proportion 10 : 9. All plants within each group were self and cross incompatible with one exception. Crosses between groups were compatible with 3 exceptions. Ten plants from the reciprocal cross PIV × PIII were tested and again 2 intra-sterile inter-fertile types, GXVII and GXVIII, were found in equal numbers.

Such results by themselves could readily be explained on the *Nicotiana* type of mating system by supposing that the parent plants had one incompatibility allele in common. For example a cross such as $S1,2 \times S2,3$ would give progeny of two genotypes $S1,3$ and $S2,3$ which when intercrossed would fall into two intra-sterile inter-fertile groups as was found in *Cosmos*. Similarly the reciprocal cross $S2,3 \times S1,2$ would yield progeny with the genotypes $S1,2$ and $S1,3$.

In such a case the results of intercrossing the progeny from the reciprocal crosses and the results of crossing progeny and parents are predictable. One mating type in the progeny from each cross will be incompatible with the male parent and the other mating type will be common to both families. By making the appropriate crosses it was therefore a simple matter to show that such an explanation could not be applied to the families of *Cosmos* under discussion.

Results obtained in *Cosmos* which differ from those expected on the *Nicotiana* system are compared in table 6.

GXVI and GXVIII plants are reciprocally incompatible in *Cosmos* but so too are plants in classes GXV and GXVII, which is not so in

TABLE 6

*Comparison of the results expected from a semi-compatible cross in* Nicotiana *in which the parent plants have one allele in common and the results obtained in* Cosmos

| Cross | *NICOTIANA* Gametophytic pollen determination | | *COSMOS* Sporophytic pollen determination |
|---|---|---|---|
| | Genotypes | Results expected | Results obtained |
| PIII × PIV | $S_{1,2} \times S_{2,3}$ | +, 2 classes {GXV ($S_{1,3}$) GXVI ($S_{2,3}$)} | +, 2 classes {GXV GXVI} |
| Reciprocal | $S_{2,3} \times S_{1,2}$ | +, 2 classes {GXVII ($S_{1,2}$) GXVIII ($S_{1,3}$)} | +, 2 classes {GXVII GXVIII} |
| GXV × GXVII | $S_{1,3} \times S_{1,2}$ | + | − |
| Reciprocal | $S_{1,2} \times S_{1,3}$ | + | − |
| GXV × PIII | $S_{1,3} \times S_{1,2}$ | + | + and − |
| PIII × GXVI | $S_{1,2} \times S_{2,3}$ | + | + and − |
| PIV × GXV | $S_{2,3} \times S_{1,3}$ | + | − |
| PIV × GXVI | $S_{2,3} \times S_{2,3}$ | − | + |
| Reciprocal | $S_{2,3} \times S_{2,3}$ | − | + |

*Nicotiana*. This suggests that whichever way the cross between the parents PIII and PIV is made the resulting progeny is the same.

The two crosses GXV × PIII and PIII × GXVI show that in *Cosmos*, plants within the same groups when classified according to their behaviour with sibs, do not all behave alike when crossed with their parents, again differing from the results based on the *Nicotiana* system, and proving that there must be two genotypes within one group.

On the Composite mating system we can designate PIII as **R**7,8 and PIV as **R**1,2. The progeny from the reciprocal crosses between these two types would be **R**1,7, **R**1,8, **R**2,7, and **R**2,8 in both cases. If we assume that **R**1 is dominant to **R**7 and **R**8 in the pollen then, despite the heterogeneity of plants carrying **R**1, they would all behave alike. Similarly if **R**2 is also dominant to **R**7 and **R**8 in the pollen, plants which are genotypically **R**2,7 and **R**2,8 would behave alike.

Consequently on intercrossing the four groups within a family only two mating reactions would be observed whichever way the parents were crossed and since the genotypes in the reciprocal progenies would be the same, intercrossing between them would again show the same two reactions. This is exactly the behaviour which was observed.

Progeny from the cross PIII × PIV were intercrossed with the parents on a small scale only. The results are given in detail in table 7.

TABLE 7

*Incompatibility relations between the parents* PIII *and* PIV *and their progeny*

| ♀ \ ♂ | GXV | | GXVI | | PIII | PIV | |
|---|---|---|---|---|---|---|---|
| GXV | − | − | + | + | − | + | $\underline{R1,7}$ |
| | − | − | + | + | + | | $\underline{R1,8}$ |
| GXVI | + | + | − | − | | + | $\underline{R2,7}$ |
| | + | + | − | − | + | + | $\underline{R2,8}$ |
| PIII | + | + | + | − | − | + | $\underline{R7,8}$ |
| PIV | − | − | + | + | + | − | $\underline{R1,2}$ |
| | $\underline{R1,7}$ | $\underline{R1,8}$ | $\underline{R2,7}$ | R2,8 | R7,8 | $\underline{R1,2}$ | ♀ / ♂ |

Where alleles are known to be dominant they are underlined

If the genotypes $\underline{R1,7}$ and $\underline{R1,8}$ are allocated to Group XV we can decide arbitrarily which of these classes is incompatible with R7,8 pollen and also which of the two classes $\underline{R2,7}$ and $\underline{R2,8}$ is incompatible on R7,8 styles. The different behaviour of plants within a group in these crosses makes it possible to say with certainty that 14 of the 16 possible types of cross were made between the parents and the four incompatibility genotypes in the $F_1$. The results of these pollinations do not give the complete picture of the dominance relations between the four alleles. They do show that, in the pollen, $\underline{R1}$ is dominant to the other 3 alleles, that $\underline{R2}$ is dominant to R7 and that there is no dominance between R2 and R8. In the style $\underline{R1}$ is dominant to $\underline{R2}$ and R8 but there is individual action of alleles in $\underline{R1,7}$ styles. R7 and R8 must show individual action in the pollen or style or both, and $\underline{R2}$ is operative in $\underline{R2,8}$ and $\underline{R2,7}$ styles but we do not know whether or not it is dominant. This is the maximum amount of information we can extract from the evidence. Only the single pollination $\underline{R1,7}$ and $\underline{R1,2}$ gave a result at variance with these conclusions and this could be accounted for by an error when the cross was made.

Progeny from the reciprocal crosses between PIV and PV were intercrossed within the two families and in each case two intra-sterile,

inter-fertile mating classes only were observed. Crossing plants from the two different mating types in one family with those of the other gave exactly the same results as described in the case of PIII × PIV. Further tests were not made on these two families.

## 4. CONCLUSIONS

The breeding system in *Cosmos* is quite distinct from the *Nicotiana* system but closely resembles the type described in *Parthenium* and *Crepis*. Results can be explained on the basis of one incompatibility locus with multiple alleles. In the pollen the behaviour is determined sporophytically. In both the pollen and style the alleles show dominance or individual action according to the combination. The relation between the same alleles in the pollen and in the style sometimes varies.

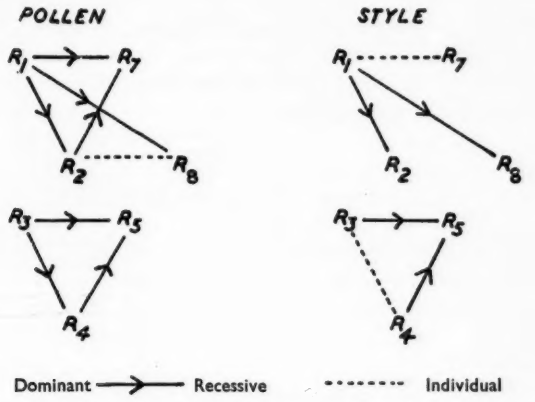


FIG. 1.—Dominance relations of two groups of alleles in the pollen and style of *Cosmos*.

The interaction of different combinations of alleles in the pollen and in the style has been worked out. The dominance relations are summed up graphically in fig. 1.

## 5. DISCUSSION

### *The mating system in* Cosmos *in relation to other types*

*Parthenium* and *Crepis* and all the types included in Class 6 (table 8) show the same genetic mechanism and all have individual gene action in the style. They are distinguished by the physiological behaviour of the pollen which is determined gametophytically in the *Œnothera* and *Nicotiana* types. But in *Parthenium* and *Crepis* the pollen control is sporophytic, some allele pairs showing dominance and others individual action. The pollen control of *Parthenium* and *Crepis* is

therefore akin to the heteromorphic class. In *Cosmos* the relationship is possibly even closer since not only is the pollen control similar, but in the style, too, dominance between alleles has been confirmed, so combining yet another character of the heteromorphic types with the genetic background of the *Nicotiana* group.

TABLE 8

*A summary of the main types of self-incompatibility in plants and their mutual relations*

| | Species | Morphology | Gene mechanics | Gene physiology | |
|---|---|---|---|---|---|
| | | | | Style | Pollen |
| 1 | *Primula* sp.<br>*Linum grandiflorum* | Heteromorphic | 1 gene, 2 alleles | Dominance | *Sporophytic control*<br>Dominance |
| 2 | *Lythrum salicaria* | ,, | 2 genes, 2 alleles | ,, | ,, |
| 3 | *Capsella grandiflora* | Homomorphic | ,, | ,, | ,, |
| 4 | *Cosmos bipinnatus* | ,, | 1 gene, multiple alleles | Dominance and Individual action | ,, |
| 5 | *Parthenium argentatum*<br>*Crepis fœtida* | ,, | ,, | Individual action | Dominance and individual action |
| 6 | *Œnothera* sp.<br>*Nicotiana* sp.<br>*Trifolium* sp.<br>*Prunus avium* | ,, | ,, | ,, | *Gametophytic control*<br>Individual action |

There is some evidence that in *Parthenium* too there may be dominance between alleles in the style. Gerstel does not mention this possibility but he found that $\mathbf{R}_{4,4}$ pollen was compatible on $\mathbf{R}_{1,4}$, $\mathbf{R}_{2,4}$ and $\mathbf{R}_{3,4}$ styles which can be explained if $\mathbf{R}_1$, $\mathbf{R}_2$ and $\mathbf{R}_3$ are dominant to $\mathbf{R}_4$ in the style as he showed them to be in the pollen.

### *Dominance*

In *C. bipinnatus* the dominance relations of alleles in the pollen sometimes present a picture of a linear series. For instance, $\mathbf{R}_3$ is dominant to $\mathbf{R}_4$ which is dominant to $\mathbf{R}_5$ and $\mathbf{R}_3$ is also dominant to $\mathbf{R}_5$. However, the whole series of alleles, which is probably extensive, does not conform to this simple pattern. There are already examples in *Cosmos*, *Crepis* and *Parthenium* of individual action of alleles in the pollen and if we consider the behaviour of some combinations

of alleles in the style of *Cosmos* we find a similar situation. Clearly therefore the linear pattern is subject to modification and it is not possible to predict the dominance relations of a pair of alleles. Furthermore their relations in the pollen and style are not necessarily the same. Complete reversal of the order of dominance in pollen and style has not been found and is unlikely since it would result in self-fertility.

## 6. SUMMARY

1. The progeny of three crosses with *Cosmos bipinnatus* all included 4 mating types.

2. Some groups within a family were cross-incompatible and there were reciprocal differences in this compatibility.

3. There was incompatibility between male and female parents and some of their progeny.

4. The results can be explained genetically by assuming that—

(*a*) there is one incompatibility locus with multiple alleles,

(*b*) the behaviour of the pollen is normally determined sporophytically and alleles exhibit dominance,

(*c*) in the style alleles show dominance or individual action according to the combination.

5. The dominance relations between pairs of alleles cannot be predicted and may be different in the pollen and style.

6. The breeding system in this species conforms in general to the Composite type described in *Parthenium* and *Crepis*. But the dominance of some alleles in the style of *Cosmos* represents a still further combination of elements of the heteromorphic and homomorphic types of breeding system.

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# POLYGENIC SYSTEMS CONTROLLING THE EXPRESSION OF MAJOR MUTANT GENES WHICH AFFECT CHAETA NUMBER IN *DROSOPHILA MELANOGASTER*

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## 1. INTRODUCTION

CONTINUOUS variation has been shown to be under the control of both genetic and environmental factors. In so far as it is genetically determined it can be accounted for by assuming the degree of expression of a character to be under the control of a number of genes of small, similar and supplementary effect. All such genes affecting any one character constitute a polygenic system, the members of which act to increase or decrease the manifestation of the character. Natural selection establishes balance in such polygenic systems by adjustment of the proportions and intra-chromosome arrangement of its members (Mather, 1942) so that the expression of the character is more or less stable and approaches the optimum for the existing environmental conditions. The polygenic systems operative in wild-type individuals will have been exposed to the continuous action of natural selection and so have become balanced in this way.

Characters showing polygenic variability may also be influenced by major genes. Since major mutants are rare in the wild, the polygenes affecting a character in their presence will not have been exposed to the balancing action of natural selection as have those of the wild type, unless the same polygenic systems are effective in the same way in the presence of either allelomorph. If this is the case, balance established in wild-type individuals will be displayed equally in the mutants, whereas if some or all members of the polygenic systems operative in the two classes differ, this parallelism will not be expected to occur.

An experiment to compare the polygenic systems operative in mutants and non-mutants was carried out by Haskell (1943). He had available four lines of *Drosophila melanogaster* with different mean numbers of abdominal chaetæ, the number of chaetæ being under polygenic control. Into each line he introduced by back-crossing the gene *scute* (*sc*), which approximately halves the number of abdominal chaetæ; this technique permitted the comparison of mutant and non-mutant brothers in each generation. If the polygenes governing the number of abdominal chaetæ are the same in mutants and non-mutants, though the gene *sc* will reduce the absolute number of abdominal chaetæ, the relative values of the line means after

back-crossing should not be affected ; while if different polygenes are operating, the relative values of the line means in the two groups will be expected to differ. Haskell found that the mean numbers of abdominal chaetæ in *scute* males did not parallel those of non-*scute* males, thus showing that the polygenes controlling chaeta number in the two groups are not the same or do not have the same action in *scute* and non-*scute* flies. This was supported by evidence of greater differences in variability of chaeta number from one generation to the next in the *scute* flies, indicating that, as might be expected from the lack of opportunity for natural selection, the polygenic systems are less balanced here than in non-*scute* flies.

Haskell's results were derived by the use of a single major gene in only four lines and his families were not very large. This same approach has been used here on a larger scale with three major mutant genes. In addition to establishing whether there was any difference between the polygenic systems operating in mutants and non-mutants, it was hoped to compare the extent of any such difference among the three mutants and also between the polygenic systems controlling two similar characters, which may be both affected by the three major mutant genes.

## 2. EXPERIMENTAL METHOD

The characters chosen for investigation were the pooled numbers of sternopleural chaetæ on the right and left sides and the pooled numbers of abdominal chaetæ on the fourth and fifth abdominal segments. These are the terminal chaeta-bearing segments in the male ; in the female the sixth segment also bears chaetæ on the ventral surface.

Ten wild-type lines of *D. melanogaster* were available, covering a wide range in their mean numbers of sternopleural and abdominal chaetæ. Of the lines, two were the inbred lines Oregon (Or.) and Samarkand (Sk.) which had been maintained by single pair brother-sister mating for 232 and 117 generations respectively when this work was begun. Six of the lines (lines 2, 3, 6, 7, 8 and 9) were among those produced by Mather and Harrison (1949) in their selection experiments. The mean chaeta numbers of the lines, obtained from counts made at intervals during the course of the experiments on stock cultures kept under conditions similar to those of the experimental flies, are given in table 1.

These six lines had been maintained in mass culture since the end of the selection experiments, a period of about three years. Since artificial selection had not been practised on the inbred lines and had been relaxed on the other six lines for three years, these eight lines will have been subject to natural selection in the culture bottle for a long period—30 to 40 generations for the selected lines. During this time the polygenic systems responsible for chaeta number will,

in so far as variation is still present in them, have been subject to the balancing action of natural selection.

Two further lines (line H and line L) were produced by selection for increased and decreased numbers of sternopleural chaetæ, selection commencing in the $F_2$ of a cross between the Oregon and Samarkand lines. Selection was at a 10 per cent. level for the first eight generations, the two male and female parents being taken from twenty on which

TABLE 1

*Mean numbers of sternopleural and abdominal chaetæ in the experimental lines*

| Line | Mean chaeta number | |
|---|---|---|
| | Sternopleurals | Abdominals |
| Sk. | ♀ 19·20 } 18·97<br>♂ 18·75 } | 37·81 } 36·35<br>34·90 } |
| Or. | ♀ 18·82 } 19·26<br>♂ 19·70 } | 42·59 } 40·76<br>38·93 } |
| 2 | ♀ 22·00 } 21·90<br>♂ 21·80 } | 35·15 } 33·63<br>32·12 } |
| 3 | ♀ 17·58 } 17·54<br>♂ 17·51 } | 44·18 } 41·32<br>41·22 } |
| 6 | ♀ 20·76 } 20·68<br>♂ 20·61 } | 67·07 } 61·36<br>55·66 } |
| 7 | ♀ 17·75 } 17·47<br>♂ 17·20 } | 52·75 } 49·05<br>45·36 } |
| 8 | ♀ 18·51 } 18·17<br>♂ 17·83 } | 56·34 } 51·29<br>46·25 } |
| 9 | ♀ 21·64 } 20·90<br>♂ 20·16 } | 54·71 } 51·61<br>48·52 } |
| H. | ♀ 25·60 } 25·15<br>♂ 24·70 } | ...<br>... |
| L. | ♀ 16·70 } 16·10<br>♂ 15·50 } | ...<br>... |

chaeta counts were made. From then on the lines were maintained in mass culture. Selection was continued at a 50 per cent. level, the ten male and female parents being taken from twenty counted. The polygenic systems responsible for chaeta number in these two lines had thus not been exposed to the action of natural selection as had those of the other eight lines, and comparisons of the variability in the two groups may not be strictly valid.

The major mutant genes investigated were *Sternopleural* (*Sp*), *Hairless* (*H*), and *scute* (*sc*) ; of these the former two are autosomal

dominants, both lethal when homozygous and *scute* is a sex-linked recessive. Details of the positions and main phenotypic effects of the mutant genes will be found in Bridges and Brehme (1944). Their effects on the sternopleural and abdominal chaetæ are shown in fig. 1.

The following methods of culture were used throughout the experiments. The flies were mated in tubes for two days, single pair matings being used in all cases. The pairs were then transferred to half-pint milk bottles, which were kept in the incubator at 25° C.

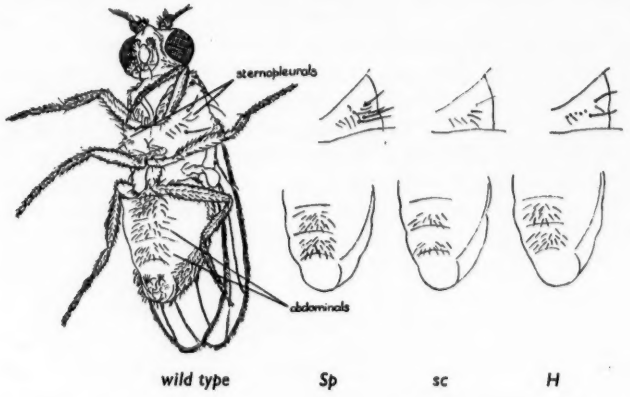


FIG. 1.—The effect of the major mutant genes *Sp*, *sc* and *H* on abdominal and sternopleural chaeta number.

The bottles of any one generation were thoroughly mixed before placing in the incubator to ensure that any temperature effects on chaeta number due to position would be at random among the different lines. The parents were shaken out from these bottles into a second set after two days, the second bottles being used in the event of failure of any of the first set to produce progeny. After emergence the flies on which counts were to be made were removed morning and evening, the sexes sorted and stored separately until counting.

The sternopleural and abdominal chaetæ were counted on twenty individuals of each sex in some generations, in others on ten ; in a few cases less than this number were used where the bottles did not yield the requisite number of flies.

## 3. CROSSING TECHNIQUE

Each of the three major mutant genes was introduced by back-crossing into certain of the test lines in such a way that mutant and non-mutant sibs were available for comparison in each generation.

The crossing technique used for *sc* differed from that for the two autosomal dominants and will be described separately.

*The autosomal dominants Sternopleural and Hairless.*—The gene *Sp* was obtained from the stock $\frac{Sp\ Bl\ L}{Cy}$ and introduced into each line individually by the necessary crosses. *H* was obtained from the stock $\frac{Cl\ B}{+}$; $\frac{Cy\ L}{Pm}$; $\frac{H}{Sb}$, and introduced into a wild-type background prior to use in these experiments, this stock, wild-type except for *H*, being used for the crosses with the test lines.

Matings were made between virgin females of the stocks containing the dominant mutant gene to be investigated and males of the test lines. Heterozygous mutant females, from the resulting $F_1$, were back-crossed to line males giving four classes of progeny, mutant and non-mutant males and females, again in the next generation. This was continued for up to 25 generations in the *Sp* experiment, 11 generations in the *H* experiment. Counts were made on chaeta number in all four classes, the females used for mating being the first three mutant females counted. Three matings were made for each line in each generation to allow for losses, the culture to be counted being taken at random from the three.

*The sex-linked recessive scute.*—This gene was obtained from the stock $\frac{Cl\ B}{sc\ v\ f\ car}$ and was introduced into a wild-type background by crossing and selection of the requisite recombinant type. The first cross made in this case was between line females and *sc* males, giving all heterozygous *scute* females (and wild-type males) in the $F_1$. Single pair matings were then made between these females and line males. In the next generation the four classes of progeny produced were $+/+$ and $+/sc$ females, which were indistinguishable phenotypically, and $+$ and *sc* males. Seven matings were made between line males and the phenotypically wild-type females. At emergence these were inspected for the presence of *scute* males in the progeny, and the culture on which counts were made was taken at random from those in which such males were found.

As it was possible to make comparisons only between mutant and non-mutant males in this experiment, counts were confined to these two classes.

Following the repeated back-crosses of the mutant females to line males, the genotypes of the experimental flies will approach more closely the genotypes of the test lines in each succeeding generation, polygenes brought in from the mutant stocks being progressively eliminated. The rate of elimination will, of course, differ for genes linked and unlinked with the major mutant. In the *n*th generation after crossing the mutant and non-mutant flies, the chance of persistence of a gene of the mutant stock will be $(1-c)^{n-1}$, where *c* is the probability

of recombination between this gene and the major mutant which is kept heterozygous (Bartlett and Haldane, 1935). For unlinked genes and linked genes with a recombination value of 50 per cent. this will reduce to $\frac{1}{2}^{n-1}$ ; for genes more tightly linked it will be less, depending on the closeness of linkage.

Thus the genetic background of mutant and non-mutant sibs will be very similar after the first few generations of back-crossing, with the exception of genes very closely linked to the major mutant, and will be similar also to that in the corresponding test lines. By the fifth back-cross, 98·4 per cent. similarity of genes of experimental and test lines will be expected for genes giving 50 per cent. recombination with the mutant gene ; this value will be less for genes giving less than 50 per cent. recombination. (A possible instance of the effect of genes closely linked to the major mutant, on abdominal chaeta number in *Sp* flies is discussed later.)

## 4. RESULTS

### (i) *The counts*

From counts made of sternopleural and abdominal chaeta number a mean value was obtained for mutants and non-mutants in each generation counted. For each of the four series (*i.e.* mutant and non-mutant abdominals and sternopleurals) in each line, an overall mean was calculated by averaging the generation means of the fifth back-cross onwards. These overall means and their standard errors are recorded in tables 2A (*Sp*), 2B (*sc*) and 2C (*H*).

It was decided to omit generations prior to the fifth back-cross (B5) in computing these overall means, since the differences between generation means were considerably more marked in the earlier generations. By B5 the genotypes of the experimental lines should be very similar to those of the corresponding test lines.

The means of the non-mutant experimental flies show little difference from those of the stock cultures kept under similar conditions (table 1). There is an apparent exception to this in the abdominals of line 9. Abdominal chaeta number in this line fell by about 15 chaetæ in the course of the experiments, from a mean of about 65 chaetæ to one of 50 in the last count made. Thus the means of the non-mutant experimental flies of line 9 in the three experiments, while they are similar to the means of counts made on the stock during the individual experiments, do not correspond with the value obtained by pooling the results of all three. The average magnitude of the effect of the major mutants on chaeta number can be seen from table 2.

A comparison of the individual mutant and non-mutant means brings out the following points. In the *Sp* mutants the abdominal chaeta number is slightly below and the sternopleural chaeta number almost double that of the corresponding non-mutant series, though

TABLE 2

*Mean chaeta number and its standard error in mutant and non-mutant flies*

TABLE 2A.—*Sp* experiment

| Line | Abdominals | | Sternopleurals | |
|---|---|---|---|---|
| | Non-mutants ($\bar{x}$) | Mutants *Sp* ($\bar{y}$) | Non-mutants ($\bar{x}$) | Mutants *Sp* ($\bar{y}$) |
| 2 | 34·63±0·36 | 32·20±0·34 | 22·23±0·27 | 37·87±0·68 |
| 3 | 42·63±0·22 | 41·91±0·21 | 17·66±0·10 | 28·34±0·38 |
| 6 | 62·91±0·57 | 60·19±0·98 | 19·69±0·20 | 33·33±0·40 |
| 7 | 51·41±0·36 | 47·06±1·02 | 17·65±0·13 | 30·27±0·25 |
| 8 | 51·88±0·47 | 46·43±0·40 | 17·35±0·19 | 31·44±0·26 |
| 9 | 59·14±1·34 | 57·63±1·11 | 19·56±0·50 | 33·68±0·57 |
| *Sk* | 35·79±0·33 | 31·85±0·43 | 19·80±0·22 | 33·40±0·74 |
| *Or* | 41·54±0·17 | 39·85±0·26 | 19·56±0·18 | 31·96±0·78 |
| *H* | 35·47±0·25 | 34·49±0·64 | 27·81±0·33 | 49·41±0·55 |
| *L* | 36·19±0·33 | 36·01±0·31 | 15·96±0·16 | 25·99±0·68 |
| Mean | 45·12 | 42·76 | 19·73 | 33·57 |
| Difference | −2·36 | | +13·84 | |

(mutant mean − non-mutant mean)

TABLE 2B.—*sc* experiment

| Line | Abdominals | | Sternopleurals | |
|---|---|---|---|---|
| | Non-mutants ($\bar{x}$) | Mutants *sc* ($\bar{y}$) | Non-mutants ($\bar{x}$) | Mutants *sc* ($\bar{y}$) |
| 2 | 31·94±0·62 | 11·19±0·53 | 23·14±0·25 | 19·17±0·17 |
| 3 | 40·72±0·23 | 20·55±0·32 | 17·67±0·24 | 15·53±0·23 |
| 6 | 57·41±0·73 | 25·50±0·34 | 19·28±0·23 | 20·04±0·54 |
| 7 | 47·20±0·36 | 22·02±0·53 | 18·00±0·15 | 17·29±0·10 |
| 8 | 47·16±0·41 | 23·00±0·38 | 17·27±0·17 | 17·32±0·16 |
| 9 | 49·27±1·51 | 21·04±0·69 | 20·74±0·30 | 19·47±0·42 |
| *Sk* | 34·63±0·24 | 15·25±0·22 | 19·46±0·21 | 17·35±0·25 |
| *Or* | 39·87±0·39 | 19·51±0·15 | 19·94±0·28 | 18·47±0·35 |
| Mean | 43·52 | 19·76 | 19·44 | 18·08 |
| Difference | −23·76 | | −1·36 | |

TABLE 2C.—*H* experiment

| Line | Abdominals | | Sternopleurals | |
|---|---|---|---|---|
| | Non-mutants ($\bar{x}$) | Mutants *H* ($\bar{y}$) | Non-mutants ($\bar{x}$) | Mutants *H* ($\bar{y}$) |
| 2 | 33·85±0·28 | 27·59±0·35 | 22·59±0·31 | 17·28±0·31 |
| 3 | 43·29±0·30 | 33·50±0·25 | 18·01±0·13 | 10·93±0·19 |
| 6 | 62·94±1·07 | 43·31±0·31 | 19·69±0·22 | 11·52±0·46 |
| 7 | 51·65±0·47 | 38·88±0·17 | 18·03±0·25 | 12·14±0·21 |
| 8 | 53·57±0·51 | 40·03±0·36 | 17·34±0·11 | 11·58±0·22 |
| 9 | 47·79±1·56 | 34·64±0·39 | 19·92±0·25 | 13·07±0·55 |
| *Or* | 41·54±0·97 | 31·93±0·77 | 19·73±0·10 | 12·41±0·43 |
| *H* | 34·81±0·37 | 27·28±0·43 | 28·41±0·27 | 21·33±0·19 |
| *L* | 35·26±0·47 | 27·58±0·42 | 15·68±0·21 | 12·52±0·17 |
| Mean | 44·97 | 33·86 | 19·93 | 13·81 |
| Difference | −11·11 | | −6·12 | |

there is no apparent change in the order of the line means for either character. The *sc* mutants have abdominal chaeta numbers considerably lower than their non-mutant brothers, and although the order of the lines is unchanged, the differences between the high lines are much smaller in mutants than in non-mutants. There is in general a slight reduction in the number of sternopleural chaetæ in *sc* mutants, the relative positions of the lines showing considerable differences from the non-mutant series, a change in the positions of lines 2 and 6 being particularly apparent.

In the *H* mutants both abdominal and sternopleural chaeta numbers are reduced. For abdominals the order of the line means is very similar in mutants and non-mutants, but for sternopleurals there are changes in the relative positions of certain lines, notably lines 6 and L.

### (ii) *Seriation*

The relative order in magnitude or seriation of the line means depends on the action of the polygenic systems. If the same polygenes are operating in mutant and non-mutant sibs, and if these have the same action in each, the relative order of the line means in mutants and non-mutants should be the same. If the polygenes differ in action in the two groups or if distinct polygenic systems are operating, the seriation of mutants and non-mutants may differ. The extent of differences in seriation will then give an indication of the extent of the differences between the operative polygenic systems of the two groups.

Comparison of the seriation was approached by the application of the regression analysis to mutant and non-mutant means. Using the values of table 2, scatter diagrams were plotted of mutant mean ($\bar{y}$) against non-mutant mean ($\bar{x}$) (Figs. 2*a* to 2*f*). If there were complete correlation between the mutant ($y$) and non-mutant ($x$) values, any increase in $y$ being proportional to an increase in $x$, an exact linear relationship would be obtained, and a straight line could be found to fit to these points, within the limits of sampling error. Where such an exact linear relationship does not exist, the points will not lie on a straight line but a " best-fitting " straight line can be found and will be that which minimises the sums of squares of the deviations of the $y$ values from their expectations, as found from the regression line. The extent to which the relation between the $x$ and $y$ values deviates from linearity will be given by the part of the total sum of squares which is attributable to the deviations of the $y$ values from the best-fitting straight line. The remainder of the sum of squares of $y$, which is removed by regression on $x$, $\left(\frac{S^2y(x-\bar{x})}{S(x-\bar{x})^2}\right)$, will indicate the extent of the similarity in seriation of mutant and non-mutant means. This value expressed as a percentage of the total sum of squares of $y$ will be termed the " percentage sum of

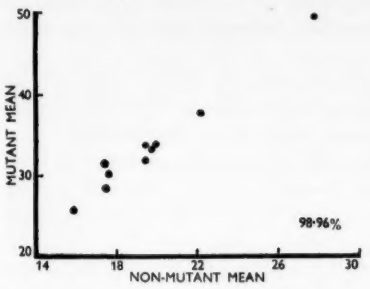


FIG. 2*a*.—Sternopleural chaetæ. *Sp* experiment.

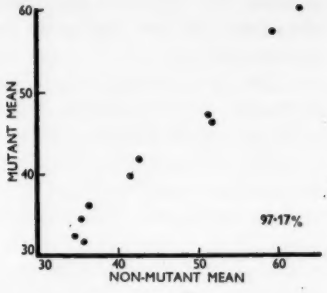


FIG. 2*d*.—Abdominal chaetæ. *Sp* experiment.

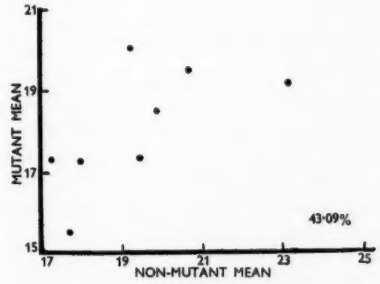


FIG. 2*b*.—Sternopleural chaetæ. *sc* experiment.

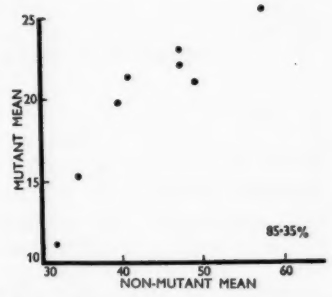


FIG. 2*e*.—Abdominal chaetæ. *sc* experiment.

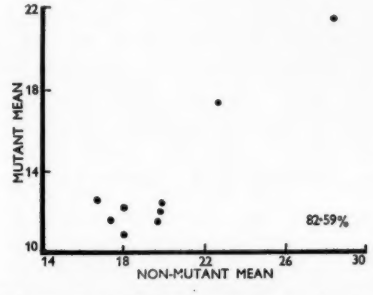


FIG. 2*c*.—Sternopleural chaetæ. *H* experiment.

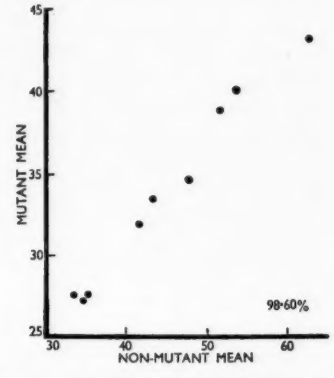


FIG. 2*f*.—Abdominal chaetæ. *H* experiment.

FIG. 2.—The relation of mean chaeta number of mutants and non-mutants.

squares of $y$ removed by regression on $x$". It affords a measure of the extent of the similarity in seriation between mutant and non-mutant means.

The percentage sum of squares of mutant means removed by regression on non-mutant means is given in table 3 for sternopleural and abdominal chaeta number in each of the three experiments.

From these results it is clear that differences exist among the three mutant genes in the extent to which the polygenic systems influencing sternopleural and abdominal chaeta number in their presence differ from those operating in their non-mutant sibs, and also that the extent of the difference is not necessarily the same for the two characters. With the gene *Sp* there is close similarity between the polygenes operating in mutants and non-mutants for both characters. With the gene *H* this similarity is shown only in respect of the abdominal

TABLE 3

*Percentage sum of squares of mutant means removed by regression on non-mutant means*

| | *Sp* | *sc* | *H* |
|---|---|---|---|
| Sternopleural chaetæ . . . | 98·96 | 43·09 | 82·59 |
| Abdominal chaetæ . . . . | 97·17 | 85·35 | 98·60 |

chaetæ, there being only 82·59 per cent. correspondence of the polygenes influencing sternopleural chaeta number, while for the gene *sc* the correspondence is even less good, 85·35 per cent. for abdominal chaetæ and only 43·09 per cent. for a sternopleural chaetæ.

Figs. 2*c* and 2*d* suggested the possibility of a curvilinear relationship between mutant and non-mutant means in the *sc* experiment for both groups of chaetæ. A quadratic regression was consequently fitted to each of these sets of points. In both cases an improvement of fit over the corresponding straight line was obtained, 59·75 per cent. of the sum of squares of $y$ being removed by the curved regression line for sternopleural chaetæ, 96·72 per cent. for abdominal chaetæ. The improvement was not significant for the sternopleurals, but was for the abdominals, giving a P very close to the 0·01 value. The possible significance of this curvilinear relationship is considered later.

### (iii) *Variability*

The variability of mutant and non-mutant flies was next investigated in order that the differences in variability between mutants and non-mutants could be compared with the divergence in seriation of the two groups. Where the sensitivity of the wild-type and mutant allelomorph is the same, that is, when they are equally susceptible to environmental variation, any differences in variability between

mutants and non-mutants with the same genetic background, may be attributed to differences in the degree of balance in the operative polygenic systems, which influence the character concerned, in the two groups. If the same polygenic systems are operating, the balance established in the test lines should be reflected in the restricted variability of both mutant and non-mutant series, the extent of variability in mutants and non-mutants being similar. If different polygenes are operating, the balance of the test-lines will not be maintained in the mutant series, and they may be expected to show an increase in variability. The comparison is, however, complicated by the possibility of differences in susceptibility of the wild-type and mutant to the effects of environmental variation, such as temperature fluctuations or even to differences in the genetic background, polygenic in the present case, which may also be considered as part of the environment of the major gene in question.

As a measure of the variability of a line in respect of each of the four series of observations (sternopleural and abdominal chaeta numbers of mutants and non-mutants) the variances of the chaeta numbers of individuals round the generation means were found and combined for each series over all generations of the line from B5 onwards : the value obtained is the overall mean variance of the series. The application of Stevens' test of homogeneity (Fabergé, 1936) to twelve sets of generation variances taken at random from each experiment showed there to be no significant deviation within the sets ; the combination of the generation variances of a series to obtain the overall mean variance is thus permissible.

In the *Sp* and *H* experiments it was necessary also to test the homogeneity of the male and female variances. The value of $z$ ($= \frac{1}{2}$ log variance ratio) was obtained from the male and female variances of the four series in each line. In finding the variance ratio the female variance was always used as numerator, the male variance as denominator, regardless of which was the larger variance ; the $z$ values thus showed negative and positive deviations from zero. To test whether the male and female variances differed significantly, the significance of the deviation of the $z$ values from zero was examined. In neither case was this significant ; the male and female variances could thus be pooled in finding the overall mean variance of a series.

To obtain the overall variance, the sum of squares and number of degrees of freedom were found for each generation in any series ; from these the total sum of squares and degrees of freedom of the series were obtained. The overall variance of the series was then found from the pooled total sums of squares and degrees of freedom, from the two sexes in the case of *H* and *Sp*, from males only in the case of *sc*. The values obtained in this way are given in table 4.

The relation between overall series variance and series mean in mutants and non-mutants is shown in the form of scatter diagrams in figs. 3*a* to 3*f*.

TABLE 4

*Overall series variances of sternopleural and abdominal chaeta number in mutant and non-mutant flies*

TABLE 4A.—*Sp* experiment

| Line | Abdominals | | Sternopleurals | |
|---|---|---|---|---|
| | Non-mutants | Mutants (*Sp*) | Non-mutants | Mutants (*Sp*) |
| 2 | 5·3786 | 6·3200 | 3·6500 | 11·8354 |
| 3 | 5·9662 | 5·5693 | 1·8263 | 8·6151 |
| 6 | 15·8138 | 13·6515 | 3·1579 | 8·9500 |
| 7 | 12·0841 | 8·9901 | 1·5859 | 8·3504 |
| 8 | 15·5625 | 10·5016 | 1·7342 | 8·3834 |
| 9 | 21·0409 | 21·6519 | 3·4305 | 11·3474 |
| *Sk* | 7·1134 | 8·0291 | 2·6206 | 12·1752 |
| *Or* | 6·6910 | 7·2717 | 2·6160 | 13·2793 |
| *H* | 7·9961 | 6·6502 | 4·4728 | 14·2636 |
| *L* | 6·3903 | 5·7410 | 1·2777 | 5·9607 |

TABLE 4B.—*sc* experiment

| Line | Abdominals | | Sternopleurals | |
|---|---|---|---|---|
| | Non-mutants | Mutants (*sc*) | Non-mutants | Mutants (*sc*) |
| 2 | 5·8776 | 2·4671 | 4·8978 | 3·0999 |
| 3 | 5·3487 | 5·7588 | 1·9037 | 2·8789 |
| 6 | 14·7060 | 6·7405 | 1·9525 | 3·1108 |
| 7 | 8·1374 | 6·5380 | 2·1415 | 2·6332 |
| 8 | 9·3376 | 7·5932 | 1·5916 | 2·1454 |
| 9 | 21·2110 | 7·9765 | 3·4072 | 3·2321 |
| *Sk* | 5·4705 | 3·3696 | 3·6144 | 1·6152 |
| *Or* | 5·4771 | 6·7381 | 2·5085 | 3·0415 |

TABLE 4C.—*H* experiment

| Line | Abdominals | | Sternopleurals | |
|---|---|---|---|---|
| | Non-mutants | Mutants (*H*) | Non-mutants | Mutants (*H*) |
| 2 | 6·0264 | 7·3616 | 3·5035 | 3·2715 |
| 3 | 4·8972 | 4·7296 | 1·9398 | 2·9611 |
| 6 | 25·9468 | 9·2623 | 2·6942 | 3·3149 |
| 7 | 11·9944 | 6·8102 | 1·5455 | 3·3000 |
| 8 | 12·4787 | 6·4241 | 1·1935 | 4·1944 |
| 9 | 26·3231 | 11·6750 | 2·3537 | 4·3296 |
| *Or* | 6·4278 | 7·0778 | 1·8899 | 3·9400 |
| *H* | 6·9977 | 4·2188 | 5·6774 | 3·1063 |
| *L* | 6·8541 | 3·7392 | 1·2731 | 2·5257 |

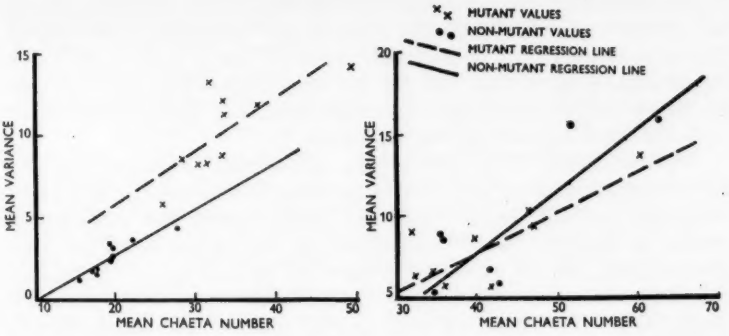


FIG. 3*a*.—Sternopleural chaetæ. *Sp* experiment. FIG. 3*d*.—Abdominal chaetæ. *Sp* experiment.

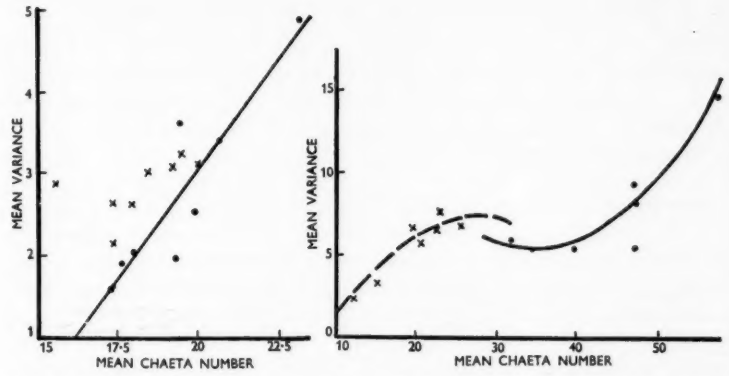


FIG. 3*b*.—Sternopleural chaetæ. *sc* experiment. FIG. 3*e*.—Abdominal chaetæ. *sc* experiment.

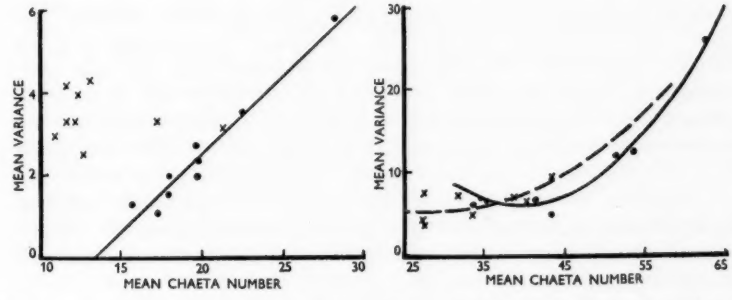


FIG. 3*c*.—Sternopleural chaetæ. *H* experiment. FIG. 3*f*.—Abdominal chaetæ. *H* experiment.

FIG. 3.—The relation of mean variance to mean chaeta number in mutants and non-mutants.

In considering the variability of abdominal chaeta number, line 9 was omitted, since the " between generations " variability of abdominal chaeta number in the non-mutant series of line 9 was found to be significantly greater than that for the other lines. This was not true for the variability of sternopleural chaeta number ; line 9 was therefore included when considering this character.

The variability of the lines for the non-mutant series, as measured by the overall variance, increased as the mean chaeta number increased. Since the mutant means frequently differed from the non-mutant means, a straightforward comparison of the variances of the two groups could not be expected to give the information required, for account must be taken of the effects of such differences on the measure of variation. Two methods were used to achieve this end, firstly the

TABLE 5

*Comparison of the coefficients of variability of mutant and non-mutant series*

| | | Mean coefficient of variability | | $t$ | N | P |
|---|---|---|---|---|---|---|
| | | Non-mutant | Mutant | | | |
| Sternopleural chaetæ | *Sp* | 0·1300 | 0·3067 | 8·6870 | 18 | $<0.001$ |
| | *H* | 0·1170 | 0·2642 | 5·3083 | 16 | $<0.001$ |
| | *sc* | 0·1382 | 0·1502 | 0·6090 | 14 | 0·5-0·7 |
| Abdominal chaetæ | *Sp* | 0·2046 | 0·1955 | 0·4289 | 16 | 0·5-0·7 |
| | *H* | 0·2148 | 0·1836 | 0·8811 | 14 | 0·3-0·5 |
| | *sc* | 0·1764 | 0·2797 | 4·2313 | 12 | 0·01-0·001 |

comparison of the coefficient of variability in corresponding mutant and non-mutant series, and secondly the method of co-variance analysis.

The coefficient of variability as measured by $\frac{\text{mean variance}}{\text{mean}}$ was found for all the lines in both mutant and non-mutant series in each experiment, and the mean coefficient of variability for each series computed. The coefficients of variability of corresponding mutant and non-mutant series were compared using a $t$ test. The coefficients of variability and the values of $t$ and P obtained are given in table 5.

It will be seen that this method brings out significant increases in variability of mutant over non-mutant in three cases—for sternopleural chaeta number in *Sp* and *H* mutants, and for abdominal chaetæ in *sc* mutants.

The method of co-variance analysis consists essentially in testing whether each set of mutant points shown in figs. 3*a* to 3*f* could be assumed to lie on the same regression line as the corresponding set of non-mutant points.

In carrying out a co-variance analysis it is not necessary actually to find the best-fitting regression lines for the mutant and non-mutant values ; these were, however, computed and included in figs. 3*a* to 3*f* to permit visual comparison of the two corresponding lines. For the data on sternopleural chaeta number in the three experiments a linear regression appeared, from the graphs, to give an adequate fit for the non-mutant values, and it was not considered necessary to use a regression of higher order. The regression of variance on mean was found to be not significant for the mutant data in the *H* and *sc* flies ; the regression lines for these data are consequently not given. The regression was, however, significant for the *Sp* mutant data, and the regression line for these data is included in fig. 3*a*. Inspection of figs. 3*d*, 3*e* and 3*f*, showing the relation of variance and mean for abdominal chaeta number, suggested that a quadratic regression line would give a better fit to the data than the linear ; both regressions were therefore computed. The quadratic regression was found to give a significant improvement in fit for the non-mutant data of the *H* and *sc* experiment and was used in preference to the linear regression. The improvement in fit for the non-mutant data of the *Sp* experiment was not significant ; the linear regression was therefore used.

In carrying out a co-variance analysis the two sets of data are pooled. From the pooled data the total and between-series sums of squares of variance and mean, and the sums of cross-products of variance and mean are computed. The difference between the two gives the within series values. A correction for regression is applied to the total and within series sums of squares for variances, removing that amount of the sums of squares attributable to regression on the mean. This correction takes up one degree of freedom where based on a linear regression, two where based on a quadratic regression. An analysis of variance is then carried out for the variance values, using the corrected total and within series sums of squares, the difference between them being the corrected between-series sum of squares. The mean squares between and within series can be compared by a *t*, found as the square root of the $\frac{\text{mean square between series}}{\text{mean square within series}}$. The number of degrees of freedom for *t* will be that of the within series item. The probability of the value of *t* obtained will show whether or not the difference between the data of the two series is significantly large (Mather, 1943*a*).

The values of *t* and P found in this way are given in table 6.

The method of co-variance analysis brings out significant differences between mutants and non-mutants in the variability of sternopleural chaeta number in both the *Sp* and *H* experiments and of abdominal chaeta number in the *sc* experiment (as did the comparison of the coefficients of variability in the two groups).

For abdominal chaeta number in the *H* and *Sp* experiments, over 97 per cent. correspondence in seriation of non-mutant and

mutant means was found. The comparison of the variances of the two groups shows that the variability of abdominal chaeta number in the *H* and *Sp* mutants is closely similar to that in the related non-mutants. In these two cases where the operative polygenic systems are similar in mutants and non-mutants, the extent of variability is, as expected, also similar.

For sternopleural chaeta number in the *Sp* experiment, where correspondence in seriation was almost 99 per cent., a significant difference in variability between mutants and non-mutants was found, there being a marked increase in variability in mutant flies. It can be seen from fig. 3*a* that this difference in variability is due almost entirely to the difference in the value of the mean for the two regressions relating variance to mean chaeta number. The direction of slope of the mutant and non-mutant regression lines is almost parallel. The regression coefficients measuring the direction

TABLE 6

*Comparison of the variability of mutant and non-mutant series*

| | | *Sp* | *sc* | *H* |
|---|---|---|---|---|
| Sternopleural chaeta number | *t* | 3·12 (17) | 1·65 (13) | 3·80 (15) |
| | P | 0·01-0·001 | 0·1-0·2 | 0·01-0·001 |
| Abdominal chaeta number | *t* | 0·38 (15) | 3·83 (10) | 0·19 (12) |
| | P | 0·7-0·8 | 0·01-0·001 | 0·8-0·9 |

Numbers in brackets refer to the degrees of freedom on which *t* is based.

of slope for the two lines were not significantly different, the non-mutant regression coefficient $b'$ being $0{\cdot}2830 \pm 0{\cdot}0469$, and the mutant regression coefficient $b''$ being $0{\cdot}3096 \pm 0{\cdot}0976$. This parallelism in the two regression lines, together with the close correspondence in the seriation of the means in mutants and non-mutants, suggests that the increase in variability is due not to the disruption of balance in mutant flies but to the greater sensitivity of the mutant phenotype to environmental fluctuations as compared with the wild-type phenotype. Since the major mutant is the same in all the lines, such an increase in variability would be expected to be of the same extent throughout, resulting in the parallel regression lines for mutants and non-mutants, as found here. The degree of expression of the mutant gene *Sp* is reported by Bridges and Brehme (1944) to be markedly influenced by temperature, the phenotype being identical with wild-type at 19° C., the degree of expression of the gene increasing as the temperature increases, at 25° C. as found in this experiment, the sternopleural chaetæ being almost double that of wild-type flies. The increase in variability in *Sp* flies then is most probably due to the

sensitivity of the *Sp* allelomorph to slight temperature differences within the culture bottle or to fluctuations in temperature in the incubator during the course of development of the flies.

Considering next those data for which more marked differences in seriation were observed, it can be seen from table 6 that in two cases, sternopleural chaeta number in the *H* experiment, with 83 per cent. correspondence in seriation of the means, and abdominal chaeta number in the *sc* experiment, with 85 per cent. correspondence, marked differences in variability in mutant and non-mutant flies were found. In the case of sternopleural chaeta number in the *sc* experiment, however, where there was only 43 per cent. correspondence in seriation of mutant and non-mutant means, co-variance analysis

TABLE 7

*Summary of results*

| Gene | Character | Average change in mean chaeta number | Per cent. correspondence in seriation of mutants and non-mutants | Effect on variability |
|---|---|---|---|---|
| *Sp* | Sternopleural chaetæ | +13·84 | 98·96 | Increased. Relation to mean unchanged |
| | Abdominal chaetæ | −2·36 | 97·17 | Unchanged |
| *sc* | Sternopleural chaetæ | −1·36 | 43·09 | Not increased. Relation to mean changed ? |
| | Abdominal chaetæ | −23·76 | 85·35 | Increased. Relation to mean changed |
| *H* | Sternopleural chaetæ | −6·12 | 82·59 | Increased. Relation to mean changed |
| | Abdominal chaetæ | −11·11 | 98·60 | Unchanged |

did not reveal any differences in variability between the two groups. It may be remembered, however, that the regression of variance on mean was not significant, while that for the non-mutant values was markedly so, indicating that, although a common regression line can be fitted to the two sets of data, there is nevertheless a difference in the relation of variability to mean in the two groups.

## 5. DISCUSSION

To facilitate discussion the results are summarised in table 7, where the gross effects of the three mutant genes on chaeta numbers, their effects on seriation and on variability are recorded.

The seriation of means of *Sp* individuals was found to be closely similar to that of non-mutants for both sternopleural and abdominal chaetæ. It may consequently be assumed that the polygenic systems

influencing these characters in the mutants are very similar to those operative in non-mutants. For the other two major genes, such a close correspondence in seriation for the two characters was not obtained ; in *H* flies the seriation was similar between mutants and non-mutants for abdominal chaetæ but not for sternopleural chaetæ. The polygenic system influencing sternopleural chaeta number in non-mutants is disturbed to a greater extent by the presence of *H* than is that for abdominal chaeta number. This is true with *sc* also, though with this gene the divergence of the polygenic systems for both characters, from that operative in non-mutants, is more marked than with the other two genes.

The extent of the divergence of the polygenic systems of mutants from those operative in non-mutants thus differs for the three major genes investigated, and in any one mutant group may differ also for the two characters, sternopleural and abdominal chaeta numbers.

While any deviation from linear relationship of mutant and non-mutant means indicates divergence in the operative polygenic systems, it cannot be determined from the available evidence whether this divergence results from differences in the mode of action in the mutant flies, of polygenes operative in non-mutants, or from the activity of newly-evoked polygenes in mutant individuals, which in the absence of the mutant allelomorph, had no differential effect upon the two characters observed. In one instance the results indicate the former to be the case. For abdominal chaetæ in *sc* individuals the line means show a close curvilinear relation (96·72 per cent. correspondence) with those in non-mutants ; there is no reason to expect any such relation where new polygenes are active—rather it would seem that, in this case, the operative polygenic system is similar in the two groups but that the presence of *sc* imposes a limitation on its activity in the lines with higher chaeta number, restricting the full activity of the members of the system making for higher chaeta number.

The polygenes investigated may be considered to be acting as modifiers, affecting the manifestation of the characters sternopleural and abdominal chaeta number. Certain of the polygenes have been shown to be operative in the same way in the mutant and non-mutant groups ; these are non-specific modifiers affecting the manifestation of the character in the presence of both wild-type and mutant allelomorphs of the major genes. Others appear to be specific modifiers, operating only in mutant or non-mutant individuals or having a different action in the two. Similar specific modifiers have been demonstrated by Fisher and Holt (1944) in the mouse. They were investigating the selective modification of the expression of the gene *Sd*, causing short tail in the heterozygous state, taillessness and early death when homozygous. By selection for increased length of tail in heterozygous *Sd* mice, a difference of over 30 mm. in tail length was obtained between the longer- and shorter-tailed mice. It was

found, however, that tail length in the homozygous non-mutant progeny was almost exactly the same in both selection lines. The modifiers having such a marked effect on tail length in the presence of the mutant allelomorph were without effect in the normal tailed mice.

Comparison of columns 3 and 4 of table 7 shows that the extent of divergence in seriation in mutants from that of non-mutants is not correlated with the magnitude of the gross effect on the mean of the mutant gene. Of particular interest are the two cases in which the mutant gene has a drastic effect on the character while leaving the seriation of the line means almost unaffected, namely, sternopleural chaeta number in *Sp* flies and abdominal chaeta number in *H* flies. The action of a major gene has been compared with that of a switch mechanism, diverting the development of the organism into one or other of the available paths, the polygenes acting as buffering genes determining the direction and precision of the developmental path taken (Mather, 1943*b*). Here then it would seem that the paths into which the development of the chaetæ is switched by the two major mutants result in marked differences from non-mutant individuals. But the polygenes delimiting the developmental paths in mutants and non-mutants are similar. Two alternatives are presented, either that the divergence of the developmental paths resulting from the action of the major mutants occurs late in development, much of the polygenic adjustment of the character occurring prior to this, or, if the major genes are presumed to act early in development, that many of the operative polygenes are common to the divergent paths followed in mutant and non-mutant individuals. Of these two alternatives, the latter would seem to be the more acceptable, since it is unlikely that so much of the fine adjustment of a character would occur prior to the action of genes of such drastic effect as *H* and *Sp*.

Mutant *Sp* individuals exhibit little difference in respect of abdominal chaetæ from their non-mutant sibs in either mean chaeta number, seriation of the means or variability. The slight reduction in mean chaeta number in *Sp* flies has probably resulted not from the direct effect of the major mutant itself but of polygenes closely linked with this locus. The evidence does not permit a decision to be made between these two alternatives, though there is some indication from line 3 that closely linked polygenes are responsible, since in several generations of this line the mutant individuals had a mean abdominal chaeta number equal to or above that of the non-mutants. This would be expected if a rare recombination very close to the *Sp* locus had occurred here, eliminating the closely-linked minus polygenes from this line. Since *Sp* affects sternopleurals markedly and abdominals only very slightly or more probably not at all, this gene must act at least sufficiently late in development for the two hair characters to have diverged.

It might be expected that between two characters as similar as sternopleural and abdominal chaeta number some obvious developmental relationship would exist, in the nature of a simple positive correlation between the two characters, both depending on the general chaeta-producing capacity of the fly, the expression of the two increasing or decreasing simultaneously, or a simple negative correlation, as would be found if there were a limited capacity for chaeta production, increase in one character resulting in a decrease in the other (Mather and Harrison, 1949).

Consideration of the gross effects of the three major mutants investigated on each of the two characters indicates that their relationship is by no means so simple. It should particularly be noticed that the mutant *Sp*, while resulting in a marked increase in sternopleural chaeta number, has only a very slight or possibly no effect on abdominal chaetæ. While they are less striking, differences in the magnitude of the effect on the two characters of the other two major genes investigated were also found. If the developmental path were much the same for the two characters, the effect of a gene on each would be expected to be similar. The observed differences in effect of each of the three major genes on sternopleural and abdominal chaetæ suggest therefore a divergence of the developmental paths of the two characters.

The differences in sternopleural chaeta number among several of the lines used had originated as a result of correlated response to selection applied to the abdominal chaetæ. Such correlated responses may be due either to pleiotropy in action of the polygenes affecting the two characters simultaneously or to linkage of the members of two distinct polygenic systems. The results of Mather and Harrison (1949) suggested that though correlated response of sternopleural and abdominal chaetæ may result in part from a physiological relation of the two characters and pleiotropy of the effective polygenes, linkage was in part responsible, though perhaps to a lesser extent. The results of the present experiments provide some further evidence on this point. The extent of the divergence of the operative polygenic systems of mutants and non-mutants differed for the two characters in the *sc* and *H* experiments. That the polygenic systems controlling the two characters are affected differently by these two major genes shows that the systems must be distinct at least in part. This is supported indirectly by the difference in the gross effects of the major gene *Sp* on the two characters, for if this gene may affect one character while having little or no effect on the other, this suggests that polygenes similarly may affect only one of the two characters.

Where the polygenic systems controlling the expression of a character are very similar in mutant and non-mutant individuals, as tested by seriation, the variability of the character, in so far as it depends on the degree of balance in the operative polygenic system, will also be expected to be similar in the two groups. Such a similarity

was found in two cases in which similarity in the operative polygenes controlling chaeta number had been demonstrated (for abdominals in the *Sp* and *H* experiments). In a third case (sternopleurals in the *Sp* experiment) there was a considerable increase in variability of the character in mutant individuals, but it should be noted that although there was here a general increase in variability throughout all the tested lines, the relation of variability to mean chaeta number remained unchanged. Where tests of seriation showed the polygenic systems of the mutants to diverge from those operative in non-mutants, the relation of variability to mean chaeta number was found to be different in the two classes, though this is not clearly brought out with *scute*. A significant increase in variability was found in two cases only, for sternopleurals in *H* individuals and for abdominals in *sc* individuals. The particular relation of variability to mean chaeta number seems then to be a property of the particular polygenic system in action, since where the polygenic systems influencing a character are similar in two groups the relation of variability to mean is similar, while where the operative polygenic systems differ the relation of variability to mean also differs.

## 6. SUMMARY

(1) Three major mutant genes affecting chaeta number were introduced by back-crossing into ten wild-type lines of *Drosophila melanogaster* having different mean numbers of sternopleural and abdominal chaetæ. The chaetæ of mutant and non-mutant flies were counted in each generation, and the counts combined to obtain the line means.

(2) The extent of correspondence in order of magnitude or seriation of the mutant and non-mutant means indicates the degree of similarity between the operative polygenic systems of the two groups.

(3) The degree of correspondence in seriation of the line means between non-mutants and mutants with the genes *Sp*, *sc* and *H* was, for abdominal chaetæ, 97·17, 85·35 and 98·60 per cent., and for sternopleural chaetæ 98·96, 43·09 and 82·59 per cent. respectively.

(4) Thus the extent of similarity in the operative polygenic systems of mutants and non-mutants differs for the different major genes and may differ also for two characters affected by the same major gene. Furthermore, the polygenic systems controlling abdominal and sternopleural chaeta number are distinct at least in part.

(5) The extent of divergence of the operative polygenic systems of mutants and non-mutants was not correlated with the magnitude of the gross effect of the mutant gene.

(6) The variabilities of the two characters were similar in mutants and non-mutants where seriation indicated that the operative polygenic

systems were similar, except in one case where the mutant phenotype is highly sensitive to environmental differences.

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# SYMBIOTIC EFFECTIVENESS IN NODULATED RED CLOVER

## I. VARIATION IN HOST AND IN BACTERIA

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## I. INTRODUCTION

THE variation to which the symbiosis of the clover root nodule bacteria (*Rhizobium trifolii* Dang.) and the leguminous host plant (*Trifolium pratense*, L.) is subject is partly of bacterial origin and partly of plant origin. It affects all phases of nodule development and function. In this series of papers attention will be directed towards the genetics of the so-called *effectiveness* of the host's response as defined by Stevens (1925) and Thornton (1939). In the effective symbiosis the nodulated plant assimilates fixed nitrogen and is therefore able to make normal growth in the complete absence of combined nitrogen in the root culture solution. In the ineffective symbiosis, on the other hand, no nitrogen is fixed in the host plant. The failure of nitrogen fixation in the ineffective nodule is thought to be due to fundamental incompatibility between bacterium and plant. This leads to premature degeneration of the infected cells of the nodule in which, presumably, nitrogen fixation would normally take place (Chen and Thornton, 1940). Intermediate degrees of effectiveness in strains of clover nodule bacteria have been described (Baldwin and Fred, 1929 and others).

Hitherto the terms effective and ineffective have been used descriptively for *strains of bacteria* according to their average symbiosis with unselected host plants of a particular species or variety. Here they will be used to describe the symbiosis itself irrespective of bacterial strain or plant type unless qualified, as for example in the original use of these terms.

It is the object of this introductory paper to distinguish between effective, intermediate, and ineffective categories of response as clearly as possible from a study of the naturally occurring variation in host plant and bacteria, and also to outline the genetic field of study. Later papers will deal with the genetic analysis of a part of this variation and with the interrelations of strain and host factors. Some of my preliminary results have been briefly reported elsewhere (Nutman, 1946*a*, 1951).

## 2. MATERIAL AND METHODS

Commercial strains of late-flowering Montgomeryshire red clover were used in all experiments. These will be referred to as unselected clover; they were obtained in the first instance from Sutton's of Reading and later from Messrs Dixon of Ware. Plants were grown in test-tube culture on a medium lacking combined nitrogen and inoculated with nodule bacteria. For details of method, see Thornton (1930) and Nutman (1949).

Response to nitrogen fixation was determined, after 100 days' growth, (1) by a visual comparison of test plants with standard plants, and (2) by taking whole plant dry weights at the end of the experiment. The standard plants for visual grading were selected afresh for each experiment and comprised the following five classes for scoring: 0, completely ineffective, indistinguishable from uninoculated control, leaves remaining small and etiolated; 1, slightly effective, youngest two or three leaves green; 2, intermediate; 3, incompletely effective; 4, normally effective. The relation of these grades to harvest dry weight is given below.

In each experiment inoculation was generally made from a subculture of a single colony picked from a plating of the bacterial strain. Details of the origin of the bacterial strains are given below. The average effectiveness of a particular bacterial strain with unselected clover was estimated by either of the two methods described above using large numbers of replicated plants, each sown singly, so that plant variability could be determined at the same time.

Unpublished data, kindly made available by Dr J. Kleczkowska and Dr H. G. Thornton, of tests of effectiveness of a large number of strains of bacteria freshly isolated from the field will also be referred to below. In these experiments each strain was tested with eight plants grown in pairs.

## 3. HOST VARIABILITY IN SYMBIOSIS

### (*a*) *With the normally effective strain of* Rhizobium trifolii, *strain A*

This strain of bacteria was originally obtained from Royal Agricultural College in Stockholm. It was effective in fixing nitrogen when first isolated and has retained this characteristic under standard conditions of subculturing in many subsequent tests (see below), so that its general stability and known history make it particularly suitable for studies on host variability. Variants have, however, arisen from time to time. The original strain lost its capacity to induce nodule formation in 1940, and a virulent line was continued in an isolate (A1) from a nodule produced by the original strain. Strain A1, and other re-isolates, have been used for comprehensive tests on strain variation (Nutman, 1946*b*) and has given rise to (i) a completely ineffective mutant (called *f* 12) after being allowed to dry out slowly in sterile soil culture (*ibid.*), and (ii) an intermediately effective variant (AC) following prolonged subculture in liquid media at 1° C. (see below).

Changes in effectiveness may also arise in culture on standard media but they do not present serious practical difficulty. I find that heterogeneity in stock cultures can be avoided by periodic plating. In this way strain A has retained the same average degree of

effectiveness with unselected clover throughout. The strain A reisolate used here has been described in previous publications as A121111.

The variation in the response of clover inoculated with strain A is shown in fig. 1 as a frequency diagram of dry weight of individual plants. This diagram combines the results of five experiments carried out at different times but under similar conditions. For plotting as a single histogram the individual dry weights for each of the five experiments were adjusted to a common mean calculated from the combined experiments.*

This diagram illustrates the very variable nature of the response of unselected clover with a pure line effective strain of nodule bacteria. The majority of plants are normally effective with a dry weight ranging from about 100 mgm. down to about 65 mgm. Plants between 65 mgm. and 30 mgm. dry weight are intermediately effective and those below 30 mgm. are wholly ineffective and are indistinguishable from uninoculated controls. The ineffective class comprises about 2 per cent. of the total population. The distribution is markedly skew with a long tail extending into classes of smaller and smaller dry weight, and appears here to be fairly continuous.

A very similar distribution has been found in a single large experiment on host-strain adaptation in which 50 strains of effective nodule bacteria were tested on nine samples of clover seed of different origin (Nutman and Read, 1952). Analysis showed that in the whole experiment average bacterial strain differences were insignificant and plant strain differences small. The histogram of the distribution of dry weight in this experiment as a whole without distinction of host or bacterial types is reproduced in fig. 2 for comparison with fig. 1. Here also the distribution is positively skew and extends over about the same range of dry weight, but it differs from fig. 1 in clearly showing a secondary mode in the ineffective class. The ineffective plants in this group were of very mixed origin and inoculated with a variety of strains, suggesting that they comprised a discrete class of plants distinguished from the remainder in giving rise to a completely ineffective symbiosis with normally effective bacterial strains.

### *(b) The response of unselected clover with the intermediately effective strains AC and 39B*

The strain AC was isolated as a variant of strain A after subculture at low temperature ; details of its origin are as follows :— Two replicated cultures maintained in liquid yeast-water mannitol medium at 1° C. from March to October 1942 gave rise on plating to colonies of two types—a large normal watery colony and a small white butyrous colony. The normal colony was much more abundant

* In figs. 1-4 the horizontal scale is divided into larger or smaller class intervals according to the amount of data available. To facilitate comparison between figures vertical scales are adjusted to give histograms of about the same maximum height.

than the variant and appeared later on the plates. The picked colonies of the normal type on re-plating gave rise at first to colonies of both types ; a second plating gave no admixture. At both platings picked colonies were tested on pairs of plants with the results shown in table 1.

Both tests showed that the parental colony type was normally effective and that the small colony variant gave rise to a highly variable response with an intermediate average. This result may be due either to an accentuation of normal host differences by the variant strain, or it may be due to further dissociation of the variant into effective and ineffective types, as has since been shown by Kleczkowska (1950) to occur in some phage resistant mutants of *Rhizobium trifolii*.

TABLE 1

*Response of trifolium plants inoculated with normal (A) and variant (AC) strains of bacteria. (Results shown in terms of number of plants in each response grade (0-4))*

| Bacterial culture | Number of plants in each grade | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| A | ... | 1 | 2 | 6 | 42 |
| AC (1st plating) | 3 | 3 | ... | 2 | 8 |
| AC (2nd plating) | 24 | ... | 2 | 4 | 4 |
| AC1 large nodule isolates | 10 | 4 | 3 | 1 | 6 |
| AC2 small nodule isolates | 3 | 2 | 3 | 3 | 1 |

The differences between the proportions of the contrasted types of response in the two platings recorded in table 1 suggest admixture but the fact that plants of like response did not usually occur together in the same tube favoured the first view.

An experiment was carried out to determine whether a mixture of effective and ineffective strains was present by re-isolating from large and from small nodules on plants in cultures containing (1) two effective plants, (2) an effective and an ineffective plant, (3) two ineffective plants. With large nodule isolates (AC1) and with small nodule isolates (AC2) similar mixtures of responses were found.

Many later tests confirmed the average intermediately effective nature of this strain ; the wide divergence in individual plant response being unrelated to further bacterial variation.

The symbiotic behaviour of this strain in terms of plant dry weight is summarised in fig. 3. This distribution differs from figs. 1 and 2 in being negatively skew, and shows a maximum frequency in the ineffective class and possibly a secondary maximum in the effective class.

A further example of an intermediate distribution, given in fig. 4,

relates to plants inoculated with strain 39B. This strain was isolated from a hill pasture on Mynydd Llangattwg, Brecknockshire, in 1943, by Dr H. G. Thornton and was shown by Dr J. Kleczkowska to be of average intermediate effectiveness in tests made in 1944 and 1945 (unpublished). The results summarised in fig. 4 confirm these findings and show further that the strain 39B in contrast to AC has a true intermediate mode at about 60 mgm., the tail of the distribution in the ineffective class containing a higher proportion of plants than with an effective strain but relatively fewer than with strain AC. The experiments made with AC and 39B summarised in figs. 3 and 4 were carried out at the same time and with the same sample of seed.

### (c) *Response of unselected clover with the ineffective bacterial strains HKC and f12*

The frequency diagram, fig. 5, shows the results of tests made with a naturally occurring ineffective strain (HKC) and a stable ineffective mutant of strain A called *f* 12, both strains previously described (Nutman, 1946*b*).

These histograms are similar in showing negative skewness with a single mode at about 14 mgm. and no secondary modes in intermediate or effective weight classes. Absolute variability is much less than with intermediate or effective strains, and probably reflects differences in the amounts or efficiency of utilisation of the original reserves of nitrogen in the seed.

## 4. BACTERIAL STRAIN VARIABILITY

Further data on bacterial strain variation is provided by extensive studies by Kleczkowska and Thornton (unpublished) on the geographical distribution in Britain of effectiveness in clover nodule bacteria, isolated from both red and white clover. In these experiments eight replicated plants of Montgomeryshire red clover were sown in pairs for each strain tested, using the agar culture technique described above. The average plant dry weight ($x$) for each strain was related to the mean dry weight of plants inoculated with the standard strain A (A) and to that of uninoculated control plants (C). The statistic $\frac{x-C}{A-C} \times 100$ enabled comparisons to be made of strains tested at different times. With the permission of the authors a histogram of the distribution of this ratio is given in fig. 6 for a large population of strains. The general form of this distribution resembles the distribution of individual dry weights of plants inoculated with effective strains of bacteria (figs. 1 and 2), in showing positive skewness and a marked secondary mode in the ineffective class. The long tail of high values is probably due to the chance occurrence of abnormally low estimates of A ; the effectiveness of A is undoubtedly

exceeded by some strains but not to the extent suggested by values up to +350 in fig. 6.

It was noted in the course of this survey that ineffective strains are more prevalent in the hill pastures of the west and north of Britain

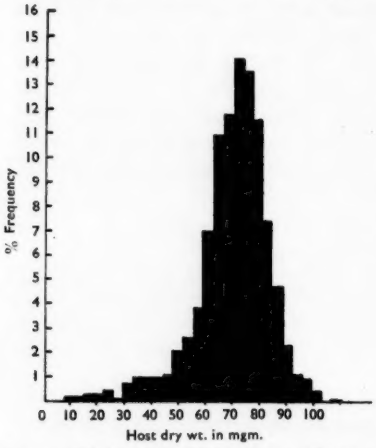


FIG. 1.—Distribution of the response of 1355 unselected clover plants inoculated with Strain A.

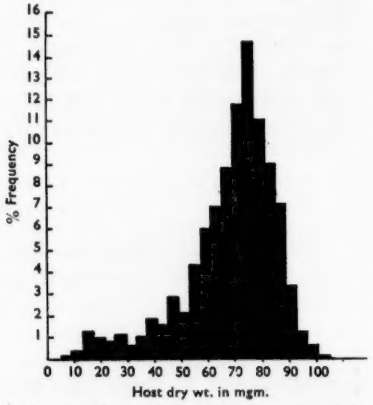


FIG. 2.—Distribution of the response of six local races of red clover inoculated with effective strains of bacteria.

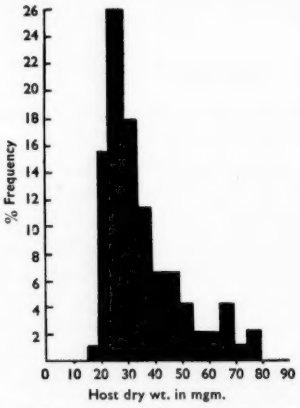


FIG. 3.—Distribution of response of red clover inoculated with the Intermediate Strain AC.

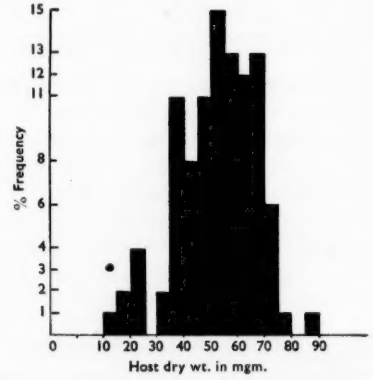


FIG. 4.—Distribution of response of red clover inoculated with the Intermediate Strain 39 B.

(Thornton, 1952) and because of the interest of these areas a large number of strains from hill pastures were examined. This has accentuated the secondary ineffective maximum. The correspondence between the histograms 1, 2 and 6 illustrates the complementary nature of the variation in host and bacteria.

The typically effective and typically ineffective types of response in symbiosis appear therefore to be separated in these diagrams by an indefinite number of intermediate grades. These may be regarded as parts of the normal distributions of effective or ineffective populations or as modal grades of a number of true intermediate distributions.

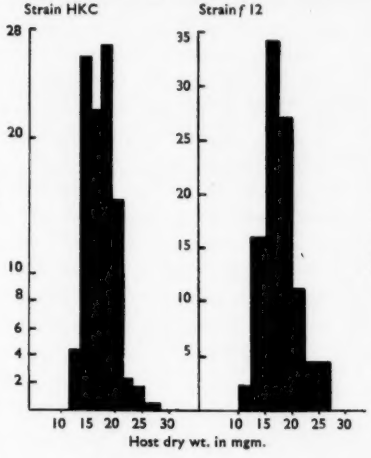


FIG. 5.—Distribution of response among clover plants inoculated with the Ineffective Strains HKC and *f* 12.

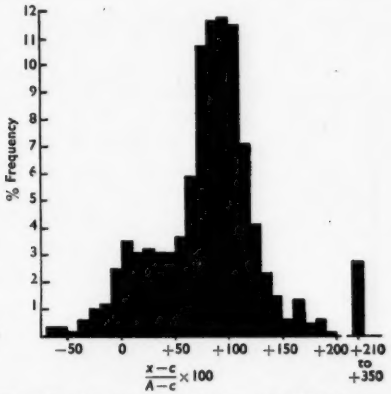


FIG. 6.—The distribution of Effectiveness among 677 strains of *Rhizobium Trifolii*.

## 5. HOST SELECTION EXPERIMENTS

Although the response of a plant may be conveniently measured by its dry weight or N content, these criteria cannot be used in breeding experiments and recourse has to be made to the visual classification of plant response into five grades (0-4) noted above. In the application of a grading method to genetic analysis of material showing segregation of effective and ineffective plants, however, a difficulty arises in classifying intermediately responding plants. This difficulty applies in particular to the assignment of plants placed in grades " 1 " and " 2 ".

If dry weight is used as the criterion, a separation of effectively from ineffectively responding plants can be fairly accurately made in figs. 2, 4 and 5 at about 30 mgm. ; plants above this weight belong to the effective population and those below to the ineffective. It was found that 95 per cent. of the plants in grade 2 and 55 per cent. of plants in grade 1 weighed more than 30 mgm. Since small numbers are usually involved, the following simple correction has been applied to the numbers of plants placed in these categories : grade 1 plants are regarded as half effective and half ineffective and grade 2 plants as wholly effective.

### (a) *Selections and tests made with strain A*

The responses of families raised from selected plants are summarised in table 2 assuming half the responses classified in grade 1 to be effective. The parent plants' responses are also indicated in the table; two of these were exceptionally effective and vigorous and were designated 4+ on the scale used.

TABLE 2

*Percentage of ineffective plants in crosses among original selections*

(Inoculated strain A throughout)

| Family number | Cross type | No. of plants | Per cent. ineffectives (and range) |
|---|---|---|---|
| 1. *Effective × effective* | | | |
| 1 | 4+ × 4+ | 19 | 5·3 |
| 2-5 | 4+ × 4 | 114 | 2·0 (0-11·5) |
| 6-25 | 4 × 4 | 385 | 1·0 (0-9·1) |
| 26-31 | 4 × 3 | 225 | 1·5 (0-4·9) |
| 32 | 4 × 3 | 25 | 16·2 |
| 2. *Effectives × ineffectives* | | | |
| 33, 34 | 4+ × 0 | 60 | 0·9 (0-1·5) |
| 35-49 | 4 × 0 | 405 | 2·1 (0-9·4) |
| 50 | 4 × 0 | 43 | 17·4 |
| 3. *Ineffectives × ineffectives* | | | |
| (i) Parent plants unrelated | | | |
| 51-78 | all 0 × 0 | 720 | 3·6 (0-9·1) |
| 79 | | 16 | 18·5 |
| 80 | | 54 | 30·6 |
| 81 | | 27 | 98·2 |
| (ii) Parent plants related | | | |
| 82-84 | all 0 × 0 | 58 | 0·9 (0-2·2) |
| 85 | | 44 | 18·2 |
| 86 | | 4 | 25·0 |
| 87 | | 28 | 39·2 |
| 88 | | 31 | 43·5 |
| 89 | | 22 | 50·0 |
| 90 | | 27 | 66·6 |
| 91 | | 15 | 83·3 |

The results show that progeny of crosses among effectively responding plants (subsequently referred to as effectives) of grades 3, 4 or 4+ as well as crosses between effectives and ineffectively responding plants (subsequently referred to as ineffectives) were generally effective. With the exception of families No. 32 and No. 50 only 2 per cent. of

a total of 1208 plants distributed in 48 families responded ineffectively. The much higher proportion of ineffectives in the two exceptional families suggest genetic segregation of recessive factors for ineffectiveness.

More definite evidence for hereditary influences is given by the progeny tests of crosses among ineffectives. These are of two kinds : between unrelated plants and between related plants. Ten of these families, 7 of them with related parents, show varying proportions of ineffectives much in excess of the few per cent. found in the unselected populations. About equal numbers of effectives and ineffectives occur in families 88 and 89 ; about three-quarters of ineffectives in families 90 and 91.

The simple proportions of ineffectives in these families suggest simple modes of inheritance ; the genotypes of the parent plants of family 81 and probably also of 91 may be similar.

The further breeding behaviour of some of these families will be discussed in later papers of this series, but it is evident from these preliminary results that, with an effective strain of bacteria, the proportion of plants showing an ineffective response may be raised by simple selection and breeding from ineffectives.

### (*b*) *Selections and tests made with intermediate bacterial strains*

A few selections were made of plants inoculated with an intermediate strain C13R (isolated by J. Kleczkowska from clover-sick Woburn soil in 1941). This strain unfortunately became avirulent before progeny tests of the host could be made with it, and was therefore substituted in these tests by strain AC. The results are shown in table 3 (1) with the response of unselected clover for comparison.

In contrast to the results with strain A, plants raised from effectively responding parents inoculated with an intermediate strain are themselves considerably more effective than those of ineffective or mixed parentage. In this experiment unselected clover has given the least effective response ; no significant difference appears in the response of families with either one or both parents ineffective.

In view of the different results obtained with intermediate and effective strains a number of families selected with strain A were re-examined with the intermediate strains AC and 39B. These families had previously been shown to respond almost wholly effectively with strain A although their parental responses were diverse (table 2). The results are given in table 3 (ii) and show that host selection against strain A has little or no effect on the average response of plants inoculated with the intermediate strains. With strain 39B there appears to be a reduction in the number of grade 4 responding plants but without an increase of ineffectively responding plants. The numbers of plants in these tests are insufficient to distinguish small trends.

TABLE 3

*Progeny tests of selected* Trifolium *families with intermediate strains of bacteria*

| Family number | Cross type (parental responses) | No. of plants | Response (per cent.) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 |
| 1. *Selected with strain C1 3R : progeny test with strain AC* | | | | | | | |
| 01-03 | 4×4 | 60 | 12 | 2 | 13 | 32 | 41 |
| 04-05 | 4×0 | 68 | 28 | 12 | 22 | 23 | 15 |
| 06 | 0×0 | 26 | 15 | 16 | 23 | 27 | 19 |
| Unselected clover | | 48 | 40 | 21 | 17 | 14 | 8 |
| 2. *Selected with strain A* | | | | | | | |
| (i) Progeny test with strain A | | | | | | | |
| 6-9 | 4×4 | 17 | 6 | 6 | ... | 6 | 82 |
| 35-40 | 4×0 | 103 | 4 | 1 | 8 | 15 | 72 |
| 51-54 | 0×0 | 49 | 2 | ... | 2 | 22 | 73 |
| (ii) Progeny test with strain AC | | | | | | | |
| 6-9 | 4×4 | 18 | 39 | 33 | 1 | 1 | 6 |
| 35-40 | 4×0 | 52 | 54 | 25 | 15 | 4 | 2 |
| 51-54 | 0×0 | 16 | 38 | 25 | 25 | 12 | ... |
| (iii) Progeny test with strain 39B | | | | | | | |
| 6-9 | 4×4 | 17 | ... | ... | 18 | 35 | 47 |
| 35-40 | 4×0 | 58 | 7 | 10 | 12 | 40 | 31 |
| 51-54 | 0×0 | 20 | ... | ... | 45 | 50 | 5 |

## 6. CONCLUSIONS

The above examination of naturally occurring variation in the symbiosis of *Rhizobium trifolii* and *Trifolium pratense* shows that effectiveness in N-fixation in the root nodules is determined by the interaction of bacterial strain and host plant factors which are independently subject to genetic variation. Moreover, different combinations of host and strain factors may have identical expression in symbiosis. Thus the ineffective symbiosis of family 81 with strain A is phenotypically indistinguishable from that of unselected clover with the *f*12 mutant of strain A. The complementary nature of the relations of plant and bacteria is also evident in the similar distributions of effectiveness in host and strain populations (figs. 1-6).

The range of genetic variation under the influence of these factors is represented diagrammatically in fig. 7. This figure also shows the genetic interrelations in the host, in an abbreviated form, the width of the lines connecting progeny and parent indicating their approximate frequency.

These results point to the possibility under certain conditions of

breeding for increased nitrogen fixation in the host. Previous studies on varietal relationships (Wilson, Burton and Bond, 1932 ; Bjälfve, 1935, etc.) and on adaptation between local races of red clover and nodule bacteria (Nutman and Read, 1952) suggest the same possibility.

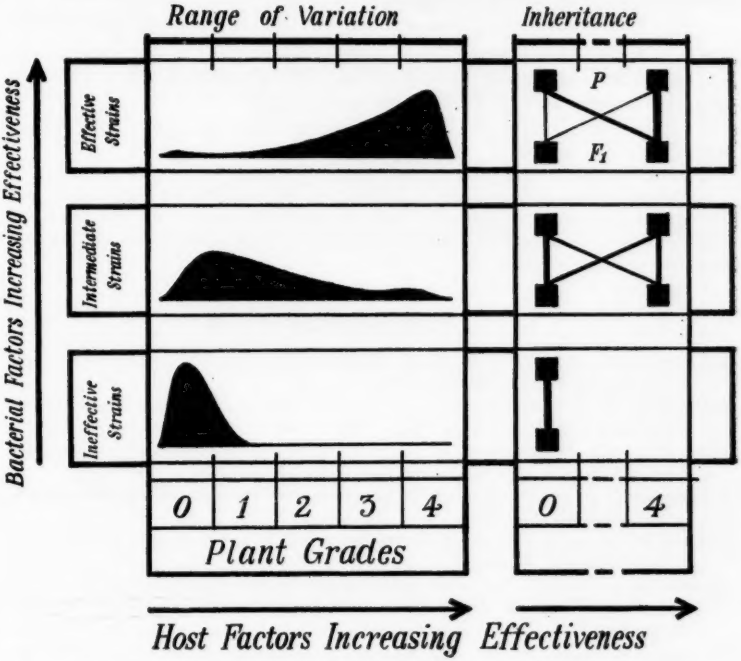


FIG. 7.—Diagram showing the genetic interrelations of host and bacteria in symbiosis. Each horizontal panel includes responses found with a single strain of bacteria. The superimposed columns show variation and inheritance in the host. The true breeding of ineffectively responding plants inoculated with ineffective strains of bacteria is inferred ; no crosses were made.

Most scope for improvement appears to lie with intermediate type symbiosis and least with those which, on the one hand, involve an ineffective bacterial strain or, on the other hand, a normally effective strain. Experiments have shown that with the latter, effectiveness is not increased by selection for a single host generation ; further experimentation on selection over a number of generations is required.

## 7. SUMMARY

From a comparative study in red clover of variation in symbiotic effectiveness, the following results were obtained :—

(1) With effective or partially effective strains of bacteria, the

response of individual plants in a variety, in terms of amount of growth made on a nitrogen-free medium showed a skew distribution.

(2) In general, distribution curves of host response with such strains show a primary mode of effectively, and a secondary mode of ineffectively responding plants.

(3) The relative sizes but not usually the positions of these modes vary with bacterial strain.

(4) With ineffective strains of bacteria the distribution curve is negatively skew and shows a single mode only.

(5) Preliminary breeding experiments show that the host plant differences are inherited.

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*Plate*

Response of clover inoculated with nodule bacteria, strain A :
(*a*) plant homozygous for $i_1$ ; (*b*) heterozygote.

# SYMBIOTIC EFFECTIVENESS IN NODULATED RED CLOVER

## II. A MAJOR GENE FOR INEFFECTIVENESS IN THE HOST

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## 1. INTRODUCTION

RESULTS reported in the preliminary paper of this series (Nutman, 1954) indicate that the sporadic ineffective response in clover inoculated with the normally effective bacterial strain A could in some cases be attributed to hereditary factors in the host. The simple proportions of effective and ineffective plants which were found to segregate in a number of families suggest simple modes of inheritance. This paper will be concerned with the subsequent breeding history of family 81, table 2 of the previous paper. This family, which was almost completely ineffective with strain A, was derived from two unrelated ineffective plants, referred to as $P_1$ and $P_2$ below.

All progeny tests were carried out under bacteriologically controlled conditions, as previously described. Each seed was planted singly in a test-tube on an agar slope inoculated either from the stock culture of strain A or from a subculture made of this strain after plating.

During each test, periodic observations were made on nodulation and growth, and at harvest, all plants were dry weighted (except those selected for further breeding) and observations made on nodulation. Visual scoring for symbiotic effectiveness in nitrogen fixation was carried out as described in the first paper ; viz. to the following grades : 0, completely ineffective ; 1, slightly effective ; 2, intermediate ; 3, incompletely effective ; 4, normally effective.

Selected plants were removed from tube culture and potted into soil and later cross-pollinated by hand, using the technique described by Williams (1925). Harvesting was done by hand and records were maintained of the numbers of florets pollinated and of the set obtained.

## 2. BREEDING EXPERIMENTS

The parent plants $P_1$ and $P_2$ were selected in 1941 from seed of different origin ; $P_1$ from the progeny of a naturally outcrossed red clover seedling and $P_2$ from a sample of late flowering Montgomeryshire red clover obtained from Messrs Sutton. Both were wholly

ineffective in their response with strain A and had large numbers of small nodules on their roots ; 68 for $P_1$ and 66 for $P_2$, compared with average numbers of nodules on effective counterparts of 20 and 28 respectively.

The results of breeding experiments with family 81 ($P_1 \times P_2$) and derivatives are set out in table 1 which shows the harvest grading of each family or group of families with strain A as inoculum throughout. In determining ratios of ineffective to effective plants in segregating families, half of the plants in grade 1 were scored as effectives and half as ineffectives (Nutman, 1954, p. 41). The data presented in this table are somewhat diverse because half-sister plants of $P_1$ were available for breeding tests. These comprised normally effective plants ($S_1$ and $S_2$) and un-nodulated resistant plants ($R_1$ and $R_2$) previously described (Nutman, 1949). Direct and reciprocal crosses are not separated in the table since no consistent maternal or paternal effects were found.

The results clearly indicate that the ineffective response in $P_1$ and $P_2$ is due to a simple Mendelian recessive, which is, however, subject to modification by other hereditary host influences.

Crosses among plants derived directly from $P_1$ and $P_2$, comprising a single self, two backcrosses and ten sib crosses, are shown in section 1 (fams. 81-104). These families are largely but not wholly ineffective ; the proportion of effectives is high in the backcrosses (approx. 1 : 1) and low in the sib crosses. The occurrence of an appreciable proportion of effectives in these families suggest a modification of the activity of the simple recessive, about which further data is given below.

The two half-sister resistant plants of $P_1$ ($R_1$ and $R_2$) with which $P_1$ and $P_2$ were crossed, did not form nodules, so that their response could not be determined. Genotypically, however, like their effective half-sisters, $S_1$ and $S_2$, the probability of their being heterozygous is high. This view is supported by the results of crosses between resistants and ineffectives : they segregated approximately equal numbers of effectives and ineffectives in two of the three families examined (fams. 105, 106), the third family (fam. 107) giving an excess of ineffectives ($\chi^2$ 3·68*).

This analysis is confirmed by the response of the progeny of crosses (i) among resistant outcross effectives (fams. 108-112), (ii) among effective segregants of families 105-106 (fams. 113-114), and (iii) between one of the effective segregants and its resistant parent (fam. 115). All of these gave one quarter ineffectives. The single cross between unlike segregants of family 105 (fam. 116) and the backcross of an ineffective to its resistant parent (fam. 117) also gave the anticipated equal numbers of effectives and ineffectives, the ineffective segregants breeding true (fams. 118, 119).

The response of resistant plants which segregated in some of these families could not be recorded, since no nodules were formed. The

TABLE 1

*Responses of families derived from the original ineffective selections $P_1$ and $P_2$ (Inoculated strain A only)*

| Family number | Cross type<br>$P_1$ and $P_2$ original ineffective selections<br>$R_1$ and $R_2$ resistant sisters of $P_1$<br>$S_1$ and $S_2$ effective sisters of $P_1$ | Grading of parents<br>4 = effective<br>0 = ineffective | Grading of progeny | | | | | Observed corrected ratios | Expected ratios | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 | | | |
| I. 81 | $P_1 \times P_2$ † | 0×0 | 26 | 1 | ... | ... | ... | ... | ... | ... |
| 92 | $P_1$ self | 0×0 | 1 | ... | ... | ... | ... | ... | ... | ... |
| 93, 94 | $P_1 \times (P_1 \times P_2)$ | 0×0 | 3 | 1 | 1 | ... | 1 | ... | ... | ... |
| 95-104 | $(P_1 \times P_2) \times (P_1 \times P_2)$ | 0×0 | 97 | 3 | 1 | 1 | 4 | ... | ... | ... |
| II. 105-107 | $P_1 \times R_1$, $P_1 \times R_2$, $P_2 \times R_2$ | 0×R | 60 | 8 | 2 | 8 | 34 | 64 : 48 | 1 : 1 | 5·32 |
| 108-115 | $R_2$ outcrosses *inter se*; $(P_1 \times R_1) \times (P_1 \times R_1)$; $R_2 \times (P_2 \times R_2)$ | 4×4 or R×4 | 25 | 11 | 5 | 16 | 75 | 30·5 : 101·5 | 1 : 3 | 2·96 |
| 116, 117 | $(P_1 \times R_1) \times (P_1 \times R_1)$; $R_2 \times (P_2 \times R_2)$ | 4×0 or R×0 | 17 | 3 | 3 | 2 | 11 | 18·5 : 17·5 | 1 : 1 | 2·95 |
| 118, 119 | $(P_1 \times R_1) \times (P_1 \times R_1)$ | 0×0 | 36 | ... | ... | 1 | ... | ... | ... | ... |
| III. 120, 121 | Outcrosses of $S_1$ and $S_2$ | 4×4 | ... | ... | ... | 2 | 62 | ... | ... | ... |
| 122-124 | $S_1$ and $S_2$ outcrosses *inter se*; $S_1 \times S_2$ | 4×4 | 41 | 8 | 17 | 16 | 73 | 45 : 110 | 1 : 3 | 2·33 |
| 125-132 | $S_1$ and $S_2$ outcrosses *inter se* | 4×4 | 1 | 3 | 14 | 41 | 44 | ... | ... | ... |
| IV. 133-136 | Outcrosses of $P_1$ and $P_2$ | 0×4 | ... | 1 | 4 | 16 | 106 | ... | ... | ... |
| 137-146 | $P_1$ and $P_2$ outcrosses *inter se* ($F_2$) | 4×4 | 26 | 1 | 7 | 30 | 71 | 26·5 : 108·5 | 1 : 3 | 15·75* |
| 147, 148 | Ineffective segregants from families 128 and 130 *inter se* | 0×0 | 61 | 6 | 1 | ... | ... | ... | ... | ... |
| V. 149, 150 | Effective recessive plant (from family 93) crossed with ineffective recessives of family 81 (see also table 6) | 4×0 | 10 | 3 | 3 | ... | 12 | 11·5 : 16·5 | 1 : 1 | 0·59 |

† Excluding expt. 2 (see table 3). Significant deviations from expectation : * P = 0·05.
Yates correction applied to small families.

presence of resistants, however, caused no disturbance in the genetic ratios. The factors concerned in the inheritance of effectiveness and resistance may therefore be considered to be independent (see also Nutman, 1949 ; table 10, p. 277).

Section III lists crosses in which two effective half-sister plants of $P_1$ are involved. These, like their resistant sisters, are also evidently heterozygous for the ineffective gene. On outcrossing, wholly effective families are obtained (fams. 120, 121) whereas in $F_2$ and in the $S_1 \times S_2$ cross (fams. 122-124) the expected proportion (one-quarter) of families segregate ineffectives, the remainder being effective (fams. 125-132).

Section IV shows that the response of outcrosses of $P_1$ or $P_2$ to unrelated effectives (fams. 133-136) is wholly effective. The expected proportion of ineffectives did not, however, occur in all the families tested in $F_2$ (fams. 137-146). The values for $\chi^2$ for the five groups of sib crosses into which these families could be divided were 3·26*, 1·59, 2·45, 1·03, 5·27*, a significant deficiency of ineffectives occurring in the last group and a significant excess in the first (consisting of one family). The ineffective segregants from two of these families bred nearly true to type (fams. 147-148).

In the material as a whole the evidence thus strongly favours the hypothesis of single gene control for ineffectiveness in the host. This gene will be designated $i_1$ and its dominant allele $I_1$.

In a minority of families there are disturbing host genetic factors affecting the simple ratios of types (*viz.* fams. 93, 94, some of fams. 95-104, fam. 107, and possibly also fam. 146). These aberrations were further investigated by crossing ineffectives of family 81 with the anomalously effective plant of family 93 (fams. 149, 150). Equal numbers of effectives and ineffectives appeared in these families suggesting that the anomalously effective parent was itself homozygous for a modifying recessive factor (provisionally designated $m_1$) which prevents the development of the ineffective response in $i_1\,i_1$ plants. On this view $i_1\,i_1\,M_1\,M_1$ or $i_1\,i_1\,M_1\,m_1$ will be ineffective and $i_1\,i_1\,m_1\,m_1$ effective. Further work is required to test this hypothesis.

## 3. DESCRIPTION OF PLANTS HOMOZYGOUS FOR $i_1$

The response of $i_1\,i_1$ plants with the otherwise effective bacterial strain A is typically ineffective and cannot be distinguished phenotypically from the response of unselected red clover in symbiosis with ineffective strains of bacteria such as strains HKC or *f*12 described in the previous paper. In both, the nodules tend to be small and numerous.

In the absence of combined nitrogen in the root medium the plants remain dwarf and N-starved although they continue to nodulate freely for some time. No increase in dry weight takes place during the test (100 days) so that at the end of this period the average dry

weight of an ineffective plant will be 15 mgm. compared with about 80 mgm. for an effective plant. Plate 1 illustrates these differences; fig. *a* shows appearance of the recessive $i_1 i_1$, and fig. *b* the heterozygote; both inoculated with strain A and segregating in the same family of plants (fam. 108).

## 4. BACTERIAL STRAIN SPECIFICITY RESPECTING $I_1$

Because of the similarity of the response of the $i_1$ line with strain A to that of unselected clover with ineffective strains of bacteria, it is of interest to examine the response of this line with bacterial strains which vary in their effectiveness with unselected clover. This was

TABLE 2

*Response of the recessive line ($i_1$ $i_1$) and unselected red clover with different strains of nodule bacteria*

| Bacterial strain | Recessive line | | | | | | Unselected clover | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Numbers of plants in each grade | | | | | Mean dry weight mgm. | Numbers of plants in each grade | | | | | Mean dry weight mgm. |
| | 0 | 1 | 2 | 3 | 4 | | 0 | 1 | 2 | 3 | 4 | |
| *Experiment* 1 | | | | | | | | | | | | |
| A | 12 | ... | ... | ... | ... | ... | ... | 1 | ... | 1 | 12 | ... |
| 2057 | ... | .. | ... | 1 | 9 | ... | ... | ... | ... | 2 | 4 | ... |
| Haldon 1 | ... | ... | 1 | ... | 7 | ... | 1 | ... | ... | ... | 3 | ... |
| Hannay 7 | ... | ... | ... | ... | 5 | ... | ... | ... | ... | ... | 5 | ... |
| Clover C1 | ... | ... | ... | ... | 6 | ... | ... | ... | ... | ... | 6 | ... |
| *Experiment* 2 | | | | | | | | | | | | |
| A | 25 | 2 | ... | 1 | 1 | 18·9 | ... | ... | 3 | ... | 3 | 34·0 |
| 37 | 13 | ... | ... | ... | ... | 16·0 | 4 | ... | ... | ... | ... | 17·5 |
| S4 | 4 | 4 | 3 | 2 | ... | 20·1 | ... | ... | 1 | 2 | 3 | 23·7 |
| 42 | 2 | 1 | 1 | 2 | ... | 22·5 | 8 | 2 | 2 | ... | 2 | 22·0 |
| 39B | 1 | 1 | 2 | 6 | 2 | 27·5 | ... | ... | 1 | 4 | ... | 25·0 |
| Sf2 | ... | ... | ... | 4 | 3 | 36·9 | ... | ... | ... | 1 | 5 | 37·4 |
| 91S2f | ... | 2 | ... | ... | 7 | 39·6 | ... | ... | ... | 1 | 5 | 31·1 |
| 49 | ... | ... | ... | ... | 12 | 42·2 | ... | ... | ... | 3 | 3 | 35·5 |

done in two experiments summarised in table 2 in terms of grading and plant dry weight. The results show that the ineffective response of the recessive line is quite specific to strain A. The response of the recessive line with other strains of bacteria of varying degrees of effectiveness with unselected clover is that appropriate to the strain. Within the limits of sampling error this relationship holds in detail for the eleven additional strains of bacteria studied; the strains falling into the same order of increasing dry weight in experiment 2 with each kind of plant material.

## 5. BACTERIAL STRAIN VARIATION

Previous studies on the stability of strain A (Nutman, 1946) showed that mutations influencing its effectiveness with unselected host material occur very rarely but when they do so they may sometimes be recognised by the larger number of smaller nodules produced. Conversely a change from ineffectiveness to effectiveness was shown by the production of larger nodules with the variant strain. Using this criterion it has been possible to detect and isolate variants of strain A which respond effectively with the recessive line; details are set out in tables 3, 4, 5 and 6.

TABLE 3

*Distribution of response in recessive line (family 81 only) inoculated with re-isolates of strain A*

| Origin of re-isolate | Name of re-isolate | Experiment | No. of plants in each grade | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 |
| Stock culture controls | Strain A | 1, Feb. 1943 | 10 | ... | ... | ... | ... |
| | | 2, June 1943 | 1 | ... | ... | 1 | 4 |
| | | 3, Nov. 1943 | 12 | ... | ... | ... | ... |
| | | 4, Apl. 1944 | 4 | 1 | ... | ... | ... |
| *Plant passage re-isolates—* | | | | | | | |
| (i) From abnormally large nodules on ineffective plants | A2 | 2 | ... | ... | 1 | 1 | 1 |
| | A3 | 2 | ... | ... | ... | 1 | 2 |
| (ii) From effective plants | A21 | 3 | ... | ... | ... | ... | 6 |
| | A21 | 4 | ... | ... | 1 | ... | 4 |
| | A31 | 3 | ... | ... | ... | 1 | 5 |
| | A211 | 4 | 1 | 1 | ... | ... | 5 |
| (iii) From small nodules on ineffective plants | A4 | 2 | 3 | ... | ... | ... | ... |
| | A5 | 2 | 3 | ... | ... | ... | ... |
| | A42 | 3 | 5 | 1 | ... | ... | ... |
| | A42 | 4 | 5 | ... | ... | ... | ... |
| | A52 | 3 | 6 | ... | ... | ... | ... |
| | A421 | 4 | 10 | ... | ... | ... | ... |

Re-isolates of the bacteria from nodules were numbered systematically, a digit being added to the parent strain number of re-isolation. Thus re-isolate A211 was taken from a nodule on a plant inoculated with re-isolate A21 which in turn derived from re-isolate A2 and strain A.

In experiments 1-4 (table 3) the recessive plant line used in tests of various re-isolates consisted of the original ineffective family $P_1 \times P_2$ (fam. 81, table 1) only. The response of unselected clover with each re-isolate was found to be normally effective and is not shown on the table.

At the first test of family 81 in February 1943, a wholly ineffective response was obtained, control clover plants giving the normal effective response. At harvest all the nodules on all the plants were measured with the results shown in table 4. Two of the plants on the recessive line had one large nodule each, whereas the remainder bore nodules of a small size only ; mean length about 0·6 mm. On unselected plants there were fewer small nodules below 1·0 mm. and a much higher proportion of nodules larger than 1·0 mm. ; the mean nodule length on individual plants ranged from 1·0 to 1·5 mm.

The two large nodules on plants 1 and 2 were of normal effective appearance and firm in texture and measured 2·2 and 3·6 mm. in

TABLE 4

*Distribution of nodule length on plants of the recessive line and on unselected red clover*

| | Number nodules per plant | Frequency distribution (per cent.) of nodules in each size group | | | |
|---|---|---|---|---|---|
| | | 0·0-0·9 mm. | 1·1·4 mm. | 1·5-1·9 mm. | above 2 mm. |
| Recessive line— | | | | | |
| Plant 1 . . . | 123 | 92·7 | 6·5 | ... | 0·8 |
| Plant 2 . . . | 111 | 93·7 | 3·6 | 1·8 | 0·9 |
| Mean remainder . . | 105 | 99·4 | 0·6 | ... | ... |
| Unselected clover . . | 46 | 63·1 | 22·9 | 11·0 | 3·0 |

length and from these the re-isolates A2 and A3 were obtained. From small nodules on the same plants, each measuring 0·5 mm. in length, the re-isolates A4 and A5 were also obtained. These were examined in experiment 2 and showed that a dissociation of strain A had taken place, the large nodule isolates giving an effective response with the recessive line and the small nodule isolates retaining the parental effective response of strain A. At this test the stock culture (which was not plated before testing) responded, however, in a largely effective manner with the recessive line. This suggests that dissociation had in fact taken place in the stock culture some time previous to experiment 1, the dissociant form having increased relative to the parent form in the interval between experiments. This possibility was examined by plating strain A and picking single colonies which were then tested in experiment 3. This test showed that the plated stock culture had now retained its completely ineffective response (for this reason the response of the recessive line in test 2 is omitted from table 1 since bacterial strain variation is here also involved).

Subsequently strain A has been plated before carrying out each group of experiments.

The stability of the variant strains A2 and A3 was further examined by successive re-isolations at random from effective plants to give the plant passage isolates A21, A31 and A211. The responses of these new re-isolates were tested on the same family of recessive line plants in experiments 3 and 4, and were found to be completely effective. The effective response of the A2 substrain was thus retained during plant passage and in subculture outside the plant. At each experiment re-isolates were also taken at random from small nodules on ineffective plants. These re-isolates, numbered A42, A52, A421 in the table,

TABLE 5

*Distribution of response in various recessive line families inoculated with strain A and re-isolates A211 and A421*

| Experiments and dates | Plant families used in tests | Distributions with | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Strain A | | | | | Re-isolate A211 | | | | | Re-isolate A421 | | | | |
| | | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| 5 and 6, 8/46-4/48 | Backcrosses fams. 93, 94 | 3 | 1 | 1 | ... | 1 | ... | ... | 1 | 2 | 5 | 15 | ... | 1 | 2 | 2 |
| 7-13, 1/45-7/52 | Sib crosses fams. 95, 104 | 60 | 1 | 1 | ... | 3 | 2 | 4 | 7 | ... | 18 | 49 | 6 | 6 | 4 | 4 |
| 14, 15, 2/52-7/52 | Recombined fam. 147 | 21 | ... | ... | ... | ... | 22 | 1 | 1 | ... | ... | 19 | 4 | 1 | ... | ... |
| 16, 1/53 | Sib crosses fams. 95, 96 | 7 | ... | ... | ... | ... | ... | 1 | 2 | 3 | 13 | ... | ... | ... | ... | ... |
| ,, | Recombined fam. 147 | 5 | ... | ... | ... | ... | 4 | 5 | 1 | ... | ... | 5 | ... | ... | ... | ... |
| ,, | fam. 148 | 5 | ... | ... | ... | ... | ... | ... | 1 | 2 | 5 | 4 | ... | ... | ... | ... |

gave rise to wholly ineffective responses in family 81 and were thus indistinguishable from strain A.

Further tests of some of these re-isolates were carried out with plant material derived from family 81. These are shown in table 5. In experiments 5 and 6 backcross families were used (fams. 93, 94, table 1). In experiments 7-13 and part of 16, tests were made of sib crosses (fams. 95-104, table 1), and in experiments 14, 15 and part of 16, families were tested in which the homozygote $i_1 i_1$ was recombined after outcrossing (fams. 147, 148, table 1).

The results of these further tests differed from those found for family 81. With backcross families the stock culture and A421 gave rise to small proportions of effective plants instead of responding wholly ineffectively. This incompletely ineffective response, however, has been noted already for these backcrosses in table 1, and was attributed to the action of modifying factors in the host ; the aberrant effectives

are probably due here to the same cause. Later experiments (7-13) undertaken with sib families in which the effect of modifying factors are less marked showed that no further change in A421 had taken place, this strain continuing to produce a largely ineffective response and the substrain A211 a largely effective response.

In experiments 14 and 15 tests were carried out on a reconstituted recessive line following outcrossing (fam. 147, table 1), and this gave completely ineffective responses throughout even with substrain A211. This change in the response of recessive line with A211 was unlikely to be due to a reversion in A211 since experiment 14 was carried out before experiments 12 and 13, and may be attributed to the use of a recombined recessive in place of one derived directly from the original parent plants. A subsequent test (expt. 16) showed that no further change had taken place in strain A211 which continued to give an effective response with the sib families 95, 96, and an ineffective response with the recombined recessive family 147, as in the previous experiment.

A closely related recombined recessive, family 148, tested at the same time gave, however, effective symbiosis with substrain A211. This family was derived from a single outcross whereas in family 147 the recessive $i_1$ gene was recombined from two outcrosses.

It would therefore appear that the interactions between bacteria and host may involve (i) a factor common to all A re-isolates reacting with $i_1$ gene, and (ii) a factor specific to strain A211 interacting with another host factor (or factors) which is lost to the $i_1$ line by outcrossing.

A number of further experiments were carried out in which isolations were made from large and small nodules appearing on plants of the recessive line, all inoculated with A421 or derivatives ; the results are summarised in table 6. All tests of re-isolates were made with direct derivative families of the original ineffective cross without outcrossing (fams. 81-104 only).

Isolations were taken from large and small nodules on plants of three kinds ; (i) ineffectively responding plants showing a markedly discontinuous distribution of nodule size, *viz.* plants with one or two large nodules and very many small ones and no nodules of intermediate size, (ii*a*) ineffective plants on which there were a number of large nodules and nodules of medium size scattered at random on the root system as well as small ones, (ii*b*) ineffective plants which produced larger nodules on the younger parts of the root ; and (iii) effectively responding plants of the recessive line with normal effective nodulation. The ineffective plants from which these isolations were made were from families 81-104 and the effectives from families 94-104.

Although no isolate was obtained similar to A211 in giving a completely effective response with the recessive line, a number of those isolated were of appreciably greater effectiveness than strain A

or the parental isolate A421 tested at the same time (table 5, experiments 7-13): *viz.* re-isolates A421641, A421643, A4211, A4212, A4219, A421, 10, A421, 11 and A421, 12. These gave rise to about equal numbers of effective and ineffective symbiosis, but their greater

TABLE 6

*Effectiveness of further re-isolates of strain A with plants homozygous for* $i_1$

Re-isolates taken from nodules on plants of families 81-104 and tested on the same families. Details given only for experiments in which re-isolates differed from strain A or from parallel re-isolates.

| Name of re-isolate | Date of isolation | Size of nodule | Numbers of plants in each response grade | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 |
| A . . . | ... | ... | 127 | 5 | 2 | 1 | 5 |
| I. *Re-isolates from nodules of two distinct sizes on ineffective plants* | | | | | | | |
| A421641 . . | 9/48 | large | 10 | 4 | 7 | 4 | 4 |
| A421642 . . | ,, | ,, | 22 | ... | ... | ... | ... |
| A421643 . . | ,, | small | 6 | 1 | 2 | 4 | 3 |
| Negative results with 12 re-isolates . . . | | | 60 | 3 | 2 | 3 | 1 |
| IIa. *Re-isolates from large nodules on younger roots of ineffective plants* | | | | | | | |
| Negative results with 2 re-isolates . . . | | | 9 | 4 | ... | ... | ... |
| IIb. *Re-isolates from ineffective plants with nodules of all sizes* | | | | | | | |
| Negative results with 5 re-isolates . . . | | | 18 | 4 | 5 | ... | ... |
| III. *Re-isolates from nodules on effective plants* | | | | | | | |
| A4211 . . | 8/46 | large | 3 | 1 | ... | ... | 2 |
| A4212 . . | ,, | ,, | 4 | ... | ... | ... | 2 |
| A4219 . . | 4/47 | ,, | 3 | 3 | 3 | 1 | ... |
| A421, 10 . . | ,, | ,, | 1 | 2 | 3 | 2 | ... |
| A4213 . . | 8/46 | small | 15 | ... | ... | ... | 1 |
| A4214 . . | ,, | ,, | 6 | ... | ... | ... | ... |
| A421, 11 . . | 4/47 | ,, | 1 | ... | 6 | ... | 2 |
| A421, 12 . . | ,, | ,, | 3 | 1 | 1 | 2 | 1 |
| Negative results with 4 re-isolates . . . | | | 33 | 4 | 1 | ... | ... |

effectiveness was not related in any consistent way to the size of nodule from which they were isolated (with the possible exceptions of A421, 11 and A421, 12) or to the host's response. Thus, while the large nodule isolate A421641 was of greater effectiveness than A, so also was the small nodule isolate A421643, whereas the companion

large nodule isolate A421642 was wholly ineffective. The absence of any exclusive influence of host response on bacterial strain variation is shown by the scattered distribution of the variant strains in the table, though they are most frequent among isolates from effective plants.

TABLE 7

*Effectiveness of A substrains on segregating lines of host and on homozygous $i_1$ lines after outcrossing*

| Family type | Distribution of response with strains indicated | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | | | | | A421, 9 and A421, 10 | | | | | A421, 11 and A421, 12 | | | | |
| | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| (i) Anomalous effectives in recessive line × ineffective parent: fams. 149, 150 | 10 | 3 | 3 | ... | 12 | ... | 1 | ... | ... | 6 | ... | 1 | 1 | 1 | 4 |
| | A | | | | | A421 | | | | | A421643 | | | | |
| (ii) Sib crosses of $F_1$ effectives after outcrossing | | | | | | | | | | | | | | | |
| Group 1, fams. 137 to 142 | 15 | ... | 3 | 11 | 55 | 23 | 1 | 1 | 16 | 37 | 1 | 5 | 6 | 22 | 42 |
| Group 2, fams. 143, 146 | 2 | ... | 1 | 11 | 14 | 8 | 4 | 3 | 7 | 9 | 5 | 6 | 6 | 8 | 6 |
| | A | | | | | A421 | | | | | A421643 | | | | |
| (iii) Sib crosses of $F_2$ ineffectives after outcrossing | | | | | | | | | | | | | | | |
| fam. 147 (derived from group 1 above) | 20 | 1 | ... | ... | ... | 21 | 2 | ... | ... | ... | 17 | 3 | 1 | ... | 1 |
| fam. 148 (derived from group 2 above) | 5 | ... | ... | ... | ... | 5 | ... | ... | ... | ... | 1 | 1 | 2 | 1 | 1 |

Material of the recessive line which had not been outcrossed was becoming exhausted at this stage, so that for the further examination of these isolates recourse was made to segregating families and to recombined recessives after outcrossing. The segregating families were of two kinds; those in which the effective response was due to the modifying factor $m_1$ (fams. 149, 150) and $F_2$ segregating families after outcrossing (fams. 137-146). The results of these tests are shown in table 7. They confirm the greater effectiveness of the substrains A421, 9, 10, 11, and 12 and A421643 compared with the ancestral strains A and A421.

With strain A each group of families gave the expected proportions of effectives and ineffectives ; those in section (i) segregating 1 : 1 and those in the section (ii) 1 : 3 (approx.) and those in the third section breeding true for ineffectiveness. With the new isolates, however, these ratios no longer held for some of the families, *viz.* for those in section (i) and the first group of sib crosses in section (ii) ; all of which responded in a largely effective manner. Group 2 families in section (ii) and the homozygote family 147 of section (iii), however, were not more effective with A421643 than with A or A421. These differences between families suggest that other host factors may also be concerned in the restoration of the effective response in the recessive line. The families in group 2, section (ii) (fams. 143-146) had already been noted above in table 1 to give rise to a *deficiency of ineffectives* with strain A which is statistically significant.

In this connection it is significant that each of the families 137-142 in group 1 of section (ii) of table 7 was derived from a pair of different outcrosses, whereas those in group 2 were in each case derived from a single outcross. Families 143-146 would therefore be genetically less heterogeneous than families 137-142. The different behaviour of these families with respect to substrain A421643 may be due to a factor contributed by the common outcross grandparent.

The largely effective response of the recombined family 148 with substrain A421643 and the failure to confirm a higher level of effectiveness in family 147 with this strain exactly parallels the results already found with these families for A211 (table 5). The effective response of $i_1$ plants with these strains depends upon the same combination of host factors.

The data is not yet extensive enough to determine the nature of these supplementary host factors, but the relatively simple proportions of effectives and ineffectives often found with these mutant strains (*e.g.* in tables 3 and 6) suggest that simple host factors may be concerned. It is, at any rate, clear that the modification of the ineffective host's response occasioned by specific mutation in the bacteria is not concerned solely with the primary gene $i_1$.

## 6. DISCUSSION

The investigation of a Mendelian recessive ($i_1$) associated with a completely ineffective response in clover inoculated with an otherwise effective strain of bacteria (strain A) has revealed a number of host-bacterial interactions of some interest.

With effective strains of bacteria other than strain A, the recessive $i_1$ line gives a normally effective response which is indistinguishable from the response of unselected material with these strains. The specific host-bacterial interaction leading to ineffectiveness in the $i_1$ line with strain A can be represented as $i_1\, i_1$/A where A denotes the bacterial strain factor involved (contributed by strain A), factors

from other strains (*e.g.* B) interacting with $i_1$ to give an effective symbiosis ($i_1\, i_1$/B).

There is also evidence from breeding experiments (table 1) that a simple modifying host factor ($m_1$) affects the response with the parent bacterial strain A so that the association of host and bacteria represented by $i_1\, i_1$, $m_1\, m_1$/A is effective, whereas $i_1\, i_1$, $M_1\, m_1$/A and $i_1\, i_1$, $M_1\, M_1$/A retain the ineffective response.

Two kinds of bacterial variant have been described. The first of these (substrain A211, table 3) restores the response of symbiosis of the constitution $i_1\, i_1$/A to complete effectiveness, resembling in phenotypic effect the combination of factors $i_1\, i_1$/B above, although it is associated here with variation in the bacterial strain. This change in effectiveness, which may be a result of a simple mutation in the bacteria (*e.g.* from A to C) appears also to be influenced by inherited factors in the host since it may no longer be detected with reconstituted $i_1$ ineffectives following outcross (table 5, family 147). Denoting these modifying host factors by N, the restored ineffective condition may be due to the following factor combination : $i_1\, i_1$, N/C.

The second type of bacterial variation indicated by the results given in table 6 is associated with the restoration of the effective response in a proportion of recessive plants only. This result again suggests that modifying plant factors O are concerned in such a way that the factor combination $i_1\, i_1$, O/D is effective.

These results can therefore all be ascribed to the combined influence of a primary gene and of modifying factors in the host interacting with hereditable bacterial factors. It is possible that each factor or group of factors in the plant interact with corresponding factors in the bacteria.

This tentative hypothesis is intended only as a pointer to future work. There is very little data on which to interrelate these factors or to determine whether they are simple or complex, dominant or recessive. The available evidence is not at variance with the view that they may be simple.

Nor is it intended, on the basis of these results, to discuss the mechanism of the genetic control of effectiveness in symbiosis. From some of these results an attempt has been made to elaborate a dynamic scheme for these interactions (Nutman, 1951), this will be revised at a later stage in these studies.

## 7. SUMMARY

1. Clover plants with a simple recessive host factor, $i_1$, show a completely ineffective response when inoculated with the normally effective bacterial strain A.

2. Plants homozygous for $i_1$ give an effective response with other effective strains of bacteria unrelated to strain A as well as with a stable variant of strain A (substrain A211).

3. Other bacterial variants of strain A were isolated which give rise to mixed responses in $i_1$ homozygotes.

4. A recessive suppressor $m_1$, is also inferred which restores $i_1 i_1$ plants to complete effectiveness with strain A.

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# GENE FREQUENCIES IN WILD POPULATIONS OF *TRIFOLIUM REPENS*

## I. DISTRIBUTION BY LATITUDE

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## I. INTRODUCTION

THE presence of hydrocyanic acid (HCN) in *Trifolium repens* L. has been realised for some time (Mirande, 1912). The plants possess cyanogenetic glucosides consisting of 80 per cent. lotaustralin and 20 per cent. linamarin (Melville and Doak, 1940) and the term glucoside is used in this paper to denote these cyanogenetic glucosides. The enzyme linamarase hydrolyses the glucoside into its components with the production of HCN.

Research has been carried out on hydrocyanic acid in *T. repens*, from three points of view : its amount in the plants (Doak, 1933 ; Rigg *et al.*, 1933 ; and Askew, 1933) ; its significance in seed certification (Foy and Hyde, 1937) ; and its possible role in contributing to bloat in ruminants (Evans and Evans, 1948). Further investigations have been concerned with the isolation of the glucoside (Melville and Doak, 1940), and of the enzyme (Coop, 1940) and their inheritance (Williams, 1939 ; Corkill, 1942 ; Atwood and Sullivan, 1943). Genetical studies (Corkill, 1942 ; Atwood and Sullivan, 1943) have shown that two independent genes determine the production of the glucoside *Ac-ac*, and enzyme *Li-li*. *T. repens* plants bearing both dominant genes crossed with the double recessive, gave a normal dihybrid segregation in $F_2$.

Previous unpublished studies made by the late R. D. Williams had shown that the percentage of cyanogenetic plants varied from 0-100 in populations of different geographical origin. The purpose of the present investigation was to determine the gene frequency in wild populations and to relate it to environmental factors. The four phenotypes :

(1) Glucoside and enzyme (*AcLi*)
(2) Glucoside only (*Acli*)
(3) Enzyme only (*acLi*)
(4) Neither glucoside nor enzyme (*acli*)

were distinguished by the picric acid test, using isolated lotaustralin and linamarase solutions as described by Corkill (1940). This technique, however, was modified by retesting the plants provisionally classified as the *Acli* phenotype with a solution of isolated lotaustralin

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to detect small amounts of enzyme, as will be described in a later paper. The Hardy-Weinberg formula ($p^2+2pq+q^2 = 1$) was applied to determine the genotypic frequencies of the populations (Hardy, 1908 ; Weinberg, 1908).

A collection of wild *T. repens* seeds was made from most European and some Near Eastern countries. This collection was made possible by the kind co-operation of the British Council, Agricultural Experimental Stations, Universities and Agricultural Ministries in the countries concerned. They were requested to adhere to the following conditions of seed collection : 20 wild *T. repens* seed heads to be collected from five different ecological areas around the named locality or " collection centre " ; the collection to be of wild *T. repens* only, and the place of collection to be well isolated from any cultivated land or reseeded pasture.

## 2. WILD *T. REPENS* POPULATIONS

Wild *T. repens* is widely distributed in Europe and Asia, where it can be found in pastures, meadows, on road sides, river banks, etc. Kousnetzoff (1926) has suggested that *T. repens* and most of the other *Trifolium* species have their centre of origin in the Mediterranean region. Wild *T. repens* has spread throughout Europe, as far as the Lake Baikal region on the mainland of Asia and to Japan and Ceylon, growing at altitudes up to 2200-2300 m. above sea level in the Alps and probably up to the same altitude in other mountain regions. *T. repens* is not known to have been indigenous outside the Old World.

### *The genetical structure of European and Near Eastern populations*

The *phenotypic structure* of populations can be expressed by the percentage frequency of the four phenotypes (*AcLi*, *Acli*, *acLi* and *acli*) and these are set out in Appendix 1. Considerable variation exists between populations in regard to their phenotypic proportions. Heterogeneity tests, using $2\times2$ contingency tables, were made to determine whether any differences existed between the phenotype frequency in samples from different geographical regions, and for this purpose the four phenotypes were grouped into two as follows : (*a*) *AcLi* phenotype ; (*b*) *Acli*, *acLi* and *acli* phenotypes.

An analysis of the population samples from geographical regions shows that the Mediterranean populations possess the highest frequency of *AcLi* phenotype. In Greece (Athens), for example, the frequency is 100 per cent., and in Israel (Huleh District), Italy (Lucca), Spain (La Coruña) the *Acli* and *acli* phenotypes occur only occasionally. Samples from northern Italy (San Daniele del Friuli, Milan), however, differ markedly from the above populations, the majority of the plants being of the *acli* type. In some of the populations from Ireland (Longford), Great Britain (Aberystwyth), France (Finistère), two to

four phenotypes are found, but in all cases the *AcLi* type predominates. The decrease in the frequency of the *AcLi* phenotype from the Mediterranean region to France is significant (heterogeneity $x^2 = 11{\cdot}62$, $P<0{\cdot}001$), as is also the decrease in frequency from France to Great Britain ($x^2 = 14{\cdot}72$, $P<0{\cdot}001$). All four phenotypes are represented in most of the German and Swiss populations (Hohenheim, Lausanne, Zürich), but the *AcLi* phenotype decreases in favour of the *Acli*, *acLi* and *acli* phenotypes ; the difference between the British and German populations is so obvious as to require no statistical proof. A Dutch

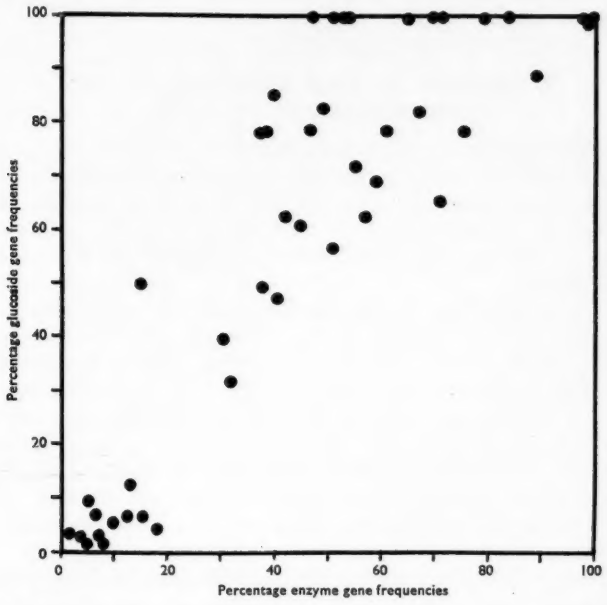


FIG. 1.—Frequencies of the dominant glucoside lotaustralin (*Ac*) and enzyme linamarase (*Li*) genes in European and Near Eastern wild populations of *Trifolium repens*.

sample (Breda) shows approximately equal proportions of the four phenotypes. The trend continues in Denmark (North Jutland), Germany (Hanover, Straubing), Austria (Rupprechtshofen), Netherlands (Groningen), where the *acli* phenotype becomes predominant. Marked trends are also noticeable in other central, eastern, and northern European samples. From the analyses it appears that the frequency of the *acli* phenotype reaches a maximum in Norwegian (Tjøtta), Hungarian (Budapest), and Polish (Kościan) samples. Unpublished data of R. D. Williams on Swedish and Russian populations indicate the existence of populations consisting entirely of acynanogenetic plants.

*Genotypic structure.*—In the investigation of the genotypic structure of populations, the proportion of dominant homozygous ($p^2$), heterozygous ($2pq$) and recessive homozygous ($q^2$) genotypes was determined by the Hardy-Weinberg formula. The genotypic frequencies of glucoside (*Ac-ac*) and enzyme genes (*Li-li*) are presented in Appendix 2.

When the glucoside and enzyme gene frequencies are plotted against one another (fig. 1) they show a gradation from very low values to extremely high values. As will be seen later, moreover, this gradation forms a close parallel with the geographical origin ranging from the low values for north-eastern Europe to the high values for the Mediterranean region.

## 3. RELATION OF GENE FREQUENCY TO THE ENVIRONMENTAL FACTORS

Marked differences have been found to occur in the genetical structure of wild *T. repens* populations from different regions in Europe and the Near East. In view of the progressive increase in the frequency of recessive genes in moving from south to north-east Europe, an attempt has been made to correlate this with environmental factors.

No correlation can be discerned between the gene frequency of populations and the rainfall of the regions in which they exist, the annual isotherms, or the July isotherm. However, it was noted that the January isotherms were very closely correlated with the gene frequency distribution. Fig. 2 shows the distribution and frequency of the glucoside gene in wild *T. repens* populations superimposed on the January isotherms in Europe and the Near East. The 46·3° F. isotherm passes through Spain, Italy, Greece and Turkey. In all samples which originated from regions near to, or on the warm side of this line, the dominant gene frequency is, in general, high. The proportion of the plants bearing the glucoside gene in the populations averages 94·8 per cent., though it reaches 100 per cent. in many cases. In the region between the 46·3° to 40·0° F. isotherms, while some samples of *T. repens* still show very high frequencies of the dominant gene, the greater number of the populations show an increase in the frequency of the recessive gene, ranging from 0 to 37·4 per cent. This area includes Great Britain, Ireland, the west and south coast of France and the northern part of Turkey. Only a few seed samples were collected in the next temperature belt from the 40·0 to 35·6° F. isotherms. These *T. repens* populations still have a high degree of dominant gene frequency. From the 35·6 to 32·0° F. lines, there is a marked change in the gene frequency pattern. A large number of samples from the Netherlands, Belgium, Germany, Switzerland, Liechtenstein and Denmark were tested and gave ample evidence of this trend towards a high rate of change in the gene frequency structure between these two isotherms. Investigations of

the gene frequency of Belgian and Dutch wild material show a continuous reduction in dominant gene frequency from Brussels (46·7 per cent.), through Breda (31·6 per cent.), to Groningen (9·3 per cent.). A similar pattern is apparent between these two isotherms in Switzerland and Liechtenstein (Lausanne, 62·4 per cent. ; Zürich,

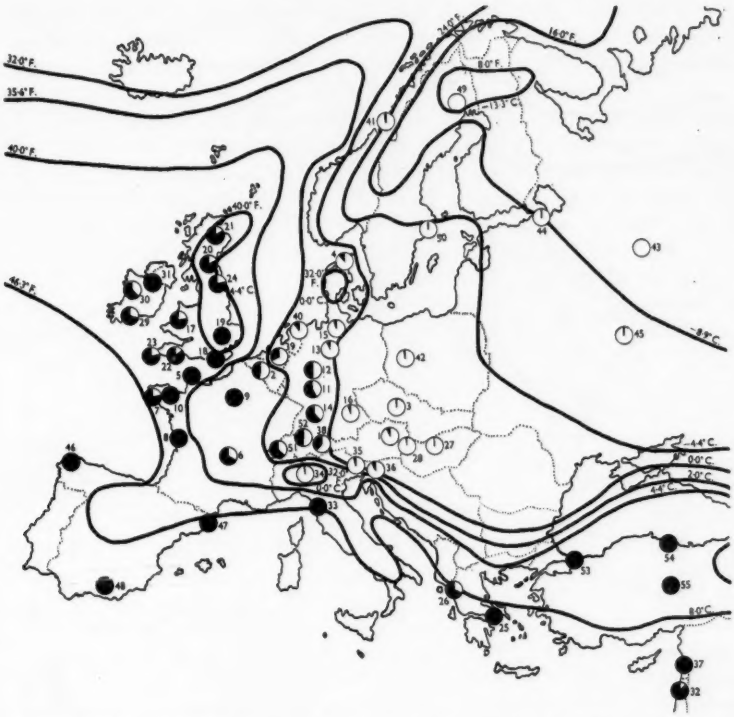


FIG. 2.—Distribution and frequency of the glucoside lotaustralin gene in European and Near Eastern wild populations of *Trifolium repens* L.

Black section : Dominant gene frequency.

White section : Recessive gene frequency.

—— January isotherm (refer to the Appendix 1 for the location numbers on the map).

49·4 per cent. ; Schaanwald, 39·4 per cent.). Striking variations were noted in the gene frequency of German populations. The three west German samples possess a high proportion of dominant genes (Hohenheim, 61·8 per cent. ; Frankfurt a/M, 57·7 per cent. ; Giessen, 50·0 per cent.), while the other two samples from locations very near the 32·0° F. isotherm have low dominant gene frequencies (Hanover, 6·4 per cent. ; Schessel, 4·3 per cent.). After crossing the 32·0° F.

isotherm the proportion of dominant genes in wild *T. repens* continues to decrease. This process, however, is much less pronounced (Germany, Straubing, 4·8 per cent. ; Austria, Rupprechtshofen, 6·5 per cent. ; Hungary, Budapest, 1·2 per cent.), than in the previous temperature belt. The 32·0° F. January isotherm occurs in the Po Valley in-

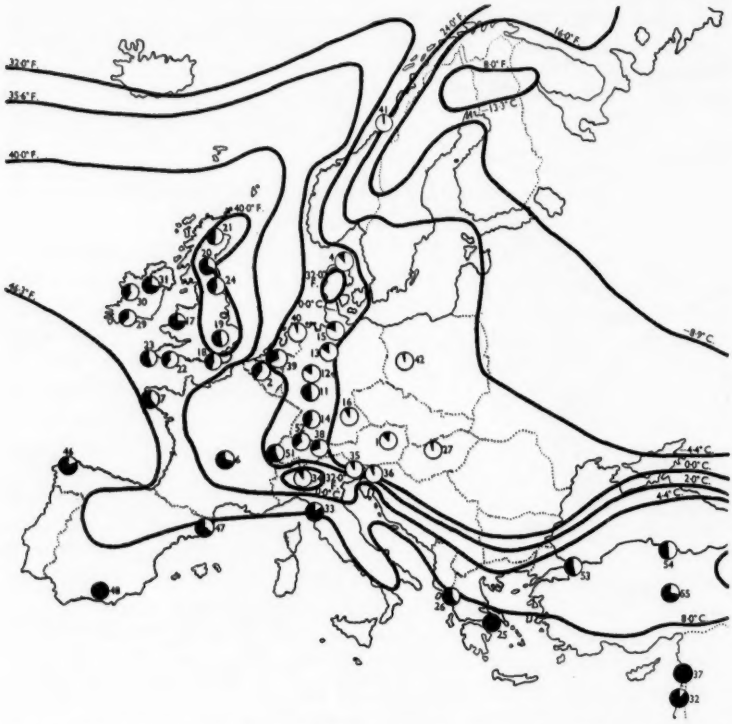


FIG. 3.—Distribution and frequency of the enzyme linamarase gene in European and Near Eastern wild populations of *Trifolium repens* L.

Black section : Dominant gene frequency.

White section : Recessive gene frequency.

—— January isotherm (refer to the Appendix 1 for the location numbers on the map).

dependently of the main isotherms across Europe. This low winter temperature seems to explain why the wild populations of the Po Valley and a cultivated strain of Ladino white clover have low percentages of dominant genes and in this respect resemble samples obtained from below the 32·0° F. line in central Europe. The lowest dominant glucoside gene frequency occurs below the 16·0° F. isotherm. No samples were obtained from Russia and Sweden for this present

investigation and the data presented have been taken from the unpublished data of R. D. Williams. According to the results of this previous investigation, in two samples out of four, cyanogenetic plants occur, but the gene frequency is reduced to very low values (U.S.S.R., Leningrad, 1·2 per cent., Orel, 0·4 per cent.). No plants with double dominant genes were found in populations from northern and eastern Europe, including Sweden (Luleå) and U.S.S.R. (Jaroslavl). A similar genetical pattern can be established in the case of the geographical distribution of the enzyme gene frequency which is illustrated in fig. 3.

The variation in the occurrence of the dominant enzyme gene is much more pronounced than that for the glucoside gene above the 40·0° F. isotherm. In the course of the investigation, populations were found with 37·9 to 100 per cent. dominant gene frequencies. The frequency of occurrence of the recessive gene was greater in Great Britain, Ireland and Turkey than in samples from the rest of the region above the 40·0° F. isotherm. In a manner similar to that of the glucoside gene, the enzyme gene composition of populations continues to alter along, and on the colder side of, the 35·6° F. isotherm. From the data relating to the dominant enzyme gene proportions, a rapid reduction towards the 32·0° F. isotherm in Belgian, Dutch, Swiss, Liechtenstein, German and Danish samples is apparent, and this conforms closely with similar reductions found in the proportions of the glucoside gene. Due to the fact that only a limited number of samples were available from the eastern half of Europe, no tests could be carried out on populations outside the 24·0° F. isotherms. Samples obtained from between the 32·0° F. and 24·0° F. isotherms have been shown to maintain the previous trend in the reduction of the dominant enzyme gene frequency and in being closely correlated with decreasing mean winter temperature.

Fig. 4 compares the mean glucoside and enzyme gene frequency of populations originating from regions between different January isotherms. It establishes a reduction in dominant allele frequencies in the successive zones, from high to low temperatures. In zones I, II and III the glucoside gene is more common than the enzyme gene. This is reversed in zone IV, where the mean frequency of the enzyme gene is more than twice as high as that of the glucoside gene. No reliable data were available concerning the enzyme gene frequency in zone V, although a greater frequency of this gene than the corresponding glucoside gene frequency is indicated by the regression coefficient.

In order to test the relationship of January temperature and the gene frequency the regression coefficient was calculated. The $t$ test showed that both of the obtained values were highly significant, being $t_{(44)} = 9{\cdot}00$ in the case of the glucoside gene frequency and $t_{(44)} = 8{\cdot}98$ in the case of the enzyme gene ($P$ in both cases less than 0·001). Both coefficients are highly significant, which demonstrates

that a close relationship exists between decreasing dominant gene frequencies and decreasing January mean temperatures. From the analyses, it was also apparent that the dominant glucoside gene frequency decreases by 4·23 and the enzyme gene frequency by 3·16 per cent. for each reduction of 1° F. (0·55° C.) January mean temperature. It is also of interest to consider whether the respective rates of decrease in glucoside and enzyme gene frequencies differ. From the analysis of variance of the decreases of the two gene frequencies it appears that the difference between the two regression

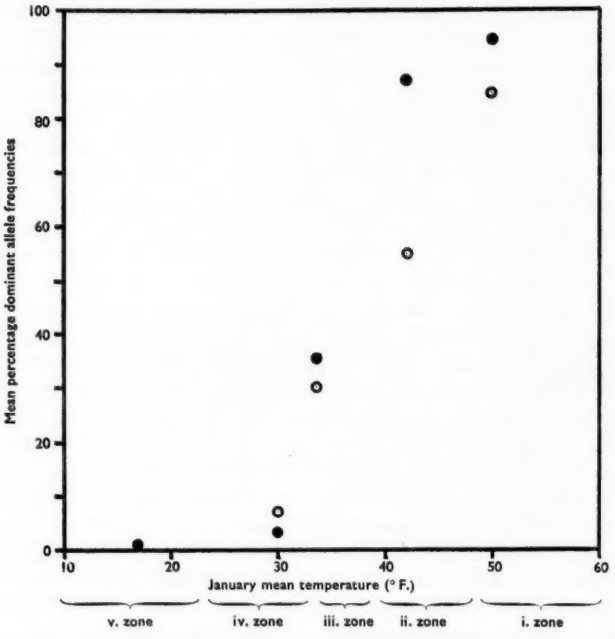


FIG. 4.—Mean frequencies of the glucoside lotaustralin (*Ac*) and enzyme linamarase (*Li*) alleles in different January temperature zones. (● = glucoside allele ; ⊙ = enzyme allele.)

coefficients is significant ($t_{(86)} = 2{\cdot}206$ with a probability of between 0·05-0·02), which suggests that the glucoside gene frequency decreases by a significantly greater amount than does the enzyme gene frequency.

## 4. GEOGRAPHICAL VARIATION AND EVOLUTION

Several authors have discussed the problem of geographical divergence, especially in human and animal populations. Boyd (1939) and Lundman (1948) studied the geographical distribution and frequency of the blood groups in man. Crampton (1916, 1932)

showed the variation of the dextral and sinistral spirals of *Partula suturalis vexillum*. The frequency of white females in the orange race of *Colias chrysotheme* (= *eurytheme*) showed a change from 74 per cent. in the North San Joaquin Valley to 13 per cent. in the Imperial Valley (Hovanitz, 1944). Dobzhansky (1939, 1944) found differences in the incidence of gene arrangements in the third chromosome among populations of *Drosophila pseudoobscura* and *Drosophila persimilis* derived from what was presumably a continuously inhabited region.

The investigations strongly indicate that geographical factors (climate, solar intensity, day-length, etc.) generally play an important role in geographical speciation. It has also been shown that organisms are able to adapt themselves to environmental gradients (*e.g.* latitude, altitude, temperature, etc.) and that natural selection moulds the character of corresponding adaptive clines. This relationship between organisms and their environments is expressed in the rules of Bergmann, Allen and Gloger (Goldschmidt, 1940). These state that there is a definite correlation between body size or pigmentation increases and geographical gradients. Bergmann's rule states that the body size of warm-blooded animals usually increases with decrease in temperature.

Beside the morphological characters, it became evident that the physiological character of species is also subjected to adaptive selection. Timoféeff-Ressovsky (1935) showed in his investigation that *Drosophila funebris* populations derived from western Europe are especially susceptible to both extremes of temperature ; the Russian and Siberian ones are particularly resistant, while *D. funebris* from the Mediterranean region is susceptible to cold, but resistant to heat. The investigation of the gene frequency in *T. repens* populations also shows the occurrence of gene frequency clines. The samples of Mediterranean origin possess the highest frequency of dominant glucoside and enzyme genes. A continuous decrease of the dominant gene frequency takes place in western and central Europe, the proportions becoming extremely low and even absent in north eastern Europe. The distribution of the frequency of both chemical characters, the presence or absence of which is governed by these two genes, shows an outstanding correlation with the January isotherms. From this it is apparent that these clines are caused by a geographical factor acting upon European wild *T. repens* populations, resulting in a gene frequency pattern ranging from a high dominant gene frequency to a very low one, depending on the factor pressure prevailing.

The picric acid test provides a means of assessing the amount of glucoside present (Melville *et al.*, 1940), as well as its frequency in the population sample. In Mediterranean samples the fresh leaves were found to contain a high proportion of HCN (more than 0·02 per cent.) whereas a large proportion of the northern European glucoside-containing plants produced very little HCN (0·006-0·02 per cent.).

Such quantitative differences are regarded as being due to the effects of modifying genes (Williams, 1939). Differences also occur in the quantity of enzyme produced. The large proportion of plants in the northern regions with a low output of gene products is probably due to natural selection.

Although no other characters were closely studied in this investigation, several observations indicate variability in other factors in the species. The leaf of *T. repens* usually bears a white marking. It is known that the presence of this character is governed by a single dominant gene (Atwood and Sullivan, 1943), the recessive allele producing no leaf mark. On the basis of a preliminary selection it was possible to distinguish six differently shaped leaf marks. Nearly every plant of Mediterranean *T. repens* has a leaf mark. All six shapes appeared and they were clearly marked on the leaf. The dominant gene was still abundantly evident in central Europe, but the recessive type was found to be more frequent than in the previous region and the leaf mark shape was less pronounced. The Norwegian population contained an increased proportion of the recessive type, with a very faint mark and little variation in shape. Further genetical research is necessary, particularly with regard to the genetical relationship between leaf mark types, before this character can be used in determining geographical gene frequency.

Comparisons of the length of the corolla tubes also disclose marked differences. The Mediterranean *T. repens* appeared to possess uniformly long corolla tubes. In contrast, the Norwegian type has a short corolla tube. *T. repens* is a highly self-sterile species and is generally pollinated by bees. This relationship between the plant and the bee makes an investigation by Alpatov (1929) particularly interesting. He studied the tongue length range of the honey bee in the plain of European Russia. Alpatov found the shortest tongue length in the northern bee populations and that the tongue length gradually increased in populations, as their origin moved southwards. If in *T. repens* populations the corolla tubes were found similarly to vary in size, *i.e.* gradually increasing in length from the shortest in the north to the longest type in the south, such evidence would be of considerable interest and importance, both biologically and practically.

Further differentiation can be mentioned in respect of habit of growth and flowering time. Generally speaking, the southern European *T. repens* appears as an erect type, with large leaves, thick stems and early flowering ; the northern European (Norwegian) type, on the other hand, is prostrate with smaller leaves, thinner stems and is late flowering.

Apart from the larger and more general character clines that have been discussed, wild *T. repens* can be divided into several ecotypes, each possessing features adapted to local microgeographical conditions. Previous investigators of geographical variation in the hereditable

characters of species have attributed this variation to genetical drift, adaptive selection, migration, isolation, etc.

Before considering the evolutionary mechanism of gene frequency variation in wild *T. repens* populations, the correlation between the decreasing winter temperature and the parallel increasing proportion of recessive genes should be stressed. Modern evolutionary views suggest that only a very low rate of mutation occurrence can be expected, while the importance of natural selection in the case of adaptive geographical variation has been pointed out. In the light of these modern theories, it can be expected that however the recessive mutant type arises in *T. repens* populations, the rate of frequency should be rather low. Because of the adaptive advantage of the carrier of the mutant gene under natural selection, the winter temperature pressure was an effective factor in the case of the widely distributed species *T. repens*. The process of change in the genetic structure of the species presumably continued until an equilibrium was reached between the selection pressure and the proportion of genotypes in *T. repens* in given regions. It is interesting to note that the selection pressure resulted in similar gene frequency clines for both glucoside and enzyme genes. However, the extent of the reaction to the selection pressure was not in all cases the same. In the warmer January temperature zones (zones I, II and III), a larger proportion of mutant recessive enzyme than glucoside genes was brought forward by the selection pressure ; by contrast, in zone IV, the recessive glucoside gene showed a significantly greater frequency, compared with the recessive enzyme genes, giving a balance between the gene frequency composition and the environmental gradient.

Further studies of different character clines in the species may reveal other progressive variation clines. The plastic nature of species makes the existence of other such character clines quite probable. These clines may show correlation with some geographical factor other than January isotherms, such as latitude.

The unpublished work of R. D. Williams was concerned with the determination of cyanogenetic plant proportions which correspond to *AcLi* phenotype. His method does not disclose the *Acli* and *acLi* phenotypes and it is therefore not suitable for the purpose of gene frequency calculations. Because no seed samples from Czechoslovakia, Russia and Sweden became available in time for the present investigation, the data of R. D. Williams were used for calculating purposes. Owing to the extremely low gene frequency, the limitation of the test can be neglected.

It may be concluded from the investigation, that in the wild *T. repens* populations there exists a gene frequency cline which is conditioned by the winter temperature prevailing, and that geographical speciation has given rise to several subspecies of wild *T. repens* in Europe and the Near East.

## 5. SUMMARY

1. The phenotypic and genotypic structures of wild *Trifolium repens* populations were investigated. By means of a modified picric acid test, *T. repens* plants were classified into four phenotypes (1) Glucoside and enzyme (*AcLi*) ; (2) Glucoside only (*Acli*) ; (3) Enzyme only (*acLi*) and (4) neither glucoside nor enzyme (*acli*) according to the presence of glucoside lotaustralin (*Ac-ac*) and enzyme linamarase (*Li-li*) genes in dominant or recessive conditions. The genotypic structure was determined by the Hardy-Weinberg formula

$$(p^2+2pq+q^2 = 1).$$

2. There was a continuous gradual decrease in the frequencies of glucoside lotaustralin and enzyme linamarase genes over the whole range from 100 to 0 per cent. as the source of the samples of wild *T. repens* populations moved from the Mediterranean region to north-eastern Europe.

3. The distribution of the dominant allele frequencies in the populations was closely correlated with the January isotherms.

4. A decrease of 1° F. in January mean temperature resulted in a reduction of 4·23 per cent. in the frequency of the glucoside gene, and a reduction of 3·16 per cent. in the frequency of the enzyme gene.

5. Thus January temperatures have played an important role through natural selection in the evolution of subspecies in *T. repens*.

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[*Continued on p.* 78.

## APPENDIX I

*Frequency of the four phenotypes in European and Near Eastern wild populations of* Trifolium repens (*per cent.*)

| No. | Localities | *AcLi* | *Acli* | *acLi* | *acli* | No. of plants |
|---|---|---|---|---|---|---|
| | *Austria* | | | | | |
| 1 | Rupprechtshofen | 7·8 | 4·8 | 15·5 | 71·9 | 103 |
| | *Belgium* | | | | | |
| 2 | Brussels | 43·2 | 28·4 | 21·0 | 7·4 | 81 |
| | *Czechoslovakia* | | | | | |
| 3 | Bohemia * | 3·1 | 96·9 | ... | ... | 96 |
| | *Denmark* | | | | | |
| 4 | North Jutland | 9·1 | 13·7 | 14·7 | 62·5 | 197 |
| | *France* | | | | | |
| 5 | Cherbourg | 85·7 | 14·3 | 0·0 | 0·0 | 35 |
| 6 | Clermont | 82·8 | 6·1 | 9·1 | 2·0 | 99 |
| 7 | Finistère | 80·2 | 15·1 | 4·7 | 0·0 | 106 |
| 8 | La Rochelle | 81·7 | 18·3 | 0·0 | 0·0 | 60 |
| 9 | Paris | 84·3 | 15·7 | 0·0 | 0·0 | 19 |
| 10 | St Brienc | 92·8 | 7·2 | 0·0 | 0·0 | 236 |
| | *Germany* | | | | | |
| 11 | Frankfurt a/M | 64·8 | 17·1 | 11·6 | 6·5 | 199 |
| 12 | Giessen | 17·5 | 57·5 | 10·0 | 15·0 | 40 |
| 13 | Hanover | 5·6 | 6·8 | 22·8 | 64·8 | 162 |
| 14 | Hohenheim | 60·1 | 25·3 | 8·9 | 5·7 | 158 |
| 15 | Schessel | 8·3 | 0·0 | 25·0 | 66·7 | 24 |
| 16 | Straubing | 8·3 | 1·1 | 10·4 | 80·2 | 96 |
| | *Great Britain* | | | | | |
| 17 | Aberystwyth | 90·1 | 5·1 | 4·0 | 0·8 | 640 |
| ... | Bangor | 58·8 | 36·5 | 3·5 | 1·2 | 85 |
| 18 | Brighton | 72·1 | 27·9 | 0·0 | 0·0 | 136 |
| 19 | Cambridge | 79·1 | 20·9 | 0·0 | 0·0 | 187 |
| 20 | Edinburgh | 85·9 | 10·9 | 3·2 | 0·0 | 92 |
| 21 | Nairn | 72·5 | 24·5 | 1·5 | 1·5 | 200 |
| 22 | Torquay | 64·6 | 33·3 | 0·0 | 2·1 | 48 |
| 23 | St Ives | 73·8 | 18·6 | 6·4 | 1·2 | 172 |
| 24 | Sunderland | 68·2 | 27·2 | 2·9 | 1·7 | 173 |
| | *Greece* | | | | | |
| 25 | Athens | 100·0 | 0·0 | 0·0 | 0·0 | 193 |
| 26 | Yanninan | 76·5 | 13·9 | 7·0 | 2·6 | 115 |
| | *Hungary* | | | | | |
| 27 | Budapest | 1·5 | 0·8 | 9·2 | 88·5 | 131 |
| 28 | Magyaróvár | 1·2 | 2·4 | 96·4 | ... | 83 |
| | *Ireland* | | | | | |
| 29 | Cork | 58·4 | 36·8 | 3·0 | 1·8 | 166 |
| 30 | Galway | 59·1 | 26·9 | 7·5 | 6·5 | 93 |
| 31 | Longford | 90·9 | 9·1 | 0·0 | 0·0 | 44 |
| | *Israel* | | | | | |
| 32 | Huleh District | 98·8 | 0·0 | 0·0 | 1·2 | 85 |

APPENDIX I—*continued*

| No. | Localities | *AcLi* | *Acli* | *acLi* | *acli* | No. of plants |
|---|---|---|---|---|---|---|
| | *Italy* | | | | | |
| 33 | Lucca | 97·4 | 2·6 | 0·0 | 0·0 | 76 |
| 34 | Milan | 1·8 | 1·7 | 15·8 | 80·7 | 171 |
| 35 | San Daniele del Friuli | 1·2 | 5·0 | 12·4 | 81·4 | 161 |
| | *Jugoslavia* | | | | | |
| 36 | Ljubljana | 3·0 | 10·4 | 9·6 | 77·0 | 135 |
| 37 | *Lebanon* | 100·0 | 0·0 | 0·0 | 0·0 | 118 |
| | *Liechtenstein* | | | | | |
| 38 | Schaanwald | 28·7 | 34·5 | 23·0 | 13·8 | 87 |
| | *Netherlands* | | | | | |
| 39 | Breda | 27·5 | 25·7 | 26·6 | 20·2 | 109 |
| 40 | Groningen | 0·0 | 17·7 | 9·7 | 72·6 | 62 |
| | *Norway* | | | | | |
| 41 | Tjøtta | 0·0 | 6·0 | 4·0 | 90·0 | 50 |
| | *Poland* | | | | | |
| 42 | Kościan | 3·0 | 1·0 | 5·0 | 91·0 | 100 |
| | *Russia* | | | | | |
| 43 | Jaroslavl * | 0·0 | 100·0 | | | 122 |
| 44 | Leningrad * | 2·7 | 97·3 | | | 111 |
| 45 | Orel * | 0·8 | 99·2 | | | 130 |
| | *Spain* | | | | | |
| 46 | La Coruña | 95·5 | 4·5 | 0·0 | 0·0 | 89 |
| 47 | Gerona | 87·7 | 12·3 | 0·0 | 0·0 | 73 |
| 48 | Granada | 100·0 | 0·0 | 0·0 | 0·0 | 112 |
| | *Sweden* | | | | | |
| 49 | Luleå * | 0·0 | 100·0 | | | 69 |
| 50 | Stockholm * | 1·7 | 98·3 | | | 59 |
| | *Switzerland* | | | | | |
| 51 | Lausanne | 70·6 | 15·2 | 10·9 | 3·3 | 184 |
| 52 | Zürich | 48·8 | 25·6 | 12·2 | 13·4 | 82 |
| | *Turkey* | | | | | |
| 53 | Adapazari | 78·4 | 21·6 | 0·0 | 0·0 | 65 |
| 54 | Samsun | 76·1 | 23·9 | 0·0 | 0·0 | 113 |
| 55 | Kayseri | 91·8 | 8·2 | 0·0 | 0·0 | 110 |

* From the unpublished data of the late R. D. Williams.

## APPENDIX II

*Frequency of the glucoside and enzyme genotypes in European and Near Eastern wild populations of* Trifolium repens

| Localities | Glucoside genotypes | | | Enzyme genotypes | | |
|---|---|---|---|---|---|---|
| | $p^2$ | $2pq$ | $q^2$ | $p^2$ | $2pq$ | $q^2$ |
| | *AcAc* | *Acac* | *acac* | *LiLi* | *Lili* | *lili* |
| *Austria* | | | | | | |
| Rupprechtshofen | 0·004 | 0·122 | 0·874 | 0·015 | 0·218 | 0·767 |
| *Belgium* | | | | | | |
| Brussels | 0·218 | 0·498 | 0·284 | 0·162 | 0·480 | 0·358 |
| *Denmark* | | | | | | |
| North Jutland | 0·015 | 0·213 | 0·772 | 0·016 | 0·222 | 0·762 |
| *France* | | | | | | |
| Cherbourg | 1·000 | 0·000 | 0·000 | ... | ... | ... |
| Clermont | 0·445 | 0·444 | 0·111 | 0·512 | 0·407 | 0·081 |
| Finistère | 0·613 | 0·340 | 0·047 | 0·374 | 0·475 | 0·151 |
| La Rochelle | 1·000 | 0·000 | 0·000 | ... | ... | ... |
| Paris | 1·000 | 0·000 | 0·000 | ... | ... | ... |
| St Brienc | 1·000 | 0·000 | 0·000 | ... | ... | ... |
| *Germany* | | | | | | |
| Frankfurt a/M | 0·331 | 0·488 | 0·181 | 0·264 | 0·500 | 0·236 |
| Giessen | 0·250 | 0·500 | 0·250 | 0·022 | 0·253 | 0·725 |
| Hanover | 0·004 | 0·120 | 0·876 | 0·024 | 0·260 | 0·716 |
| Hohenheim | 0·382 | 0·472 | 0·146 | 0·197 | 0·493 | 0·310 |
| Schessel | 0·001 | 0·082 | 0·917 | 0·033 | 0·300 | 0·667 |
| Straubing | 0·002 | 0·092 | 0·906 | 0·010 | 0·178 | 0·812 |
| *Great Britain* | | | | | | |
| Aberystwyth | 0·622 | 0·330 | 0·048 | 0·593 | 0·348 | 0·059 |
| Bangor | 0·613 | 0·340 | 0·047 | 0·149 | 0·474 | 0·377 |
| Brighton | 1·000 | 0·000 | 0·000 | 0·222 | 0·499 | 0·279 |
| Cambridge | 1·000 | 0·000 | 0·000 | 0·295 | 0·496 | 0·209 |
| Edinburgh | 0·671 | 0·296 | 0·033 | 0·449 | 0·442 | 0·109 |
| Nairn | 0·685 | 0·285 | 0·030 | 0·240 | 0·500 | 0·260 |
| Torquay | 0·732 | 0·247 | 0·021 | 0·164 | 0·482 | 0·354 |
| St Ives | 0·525 | 0·399 | 0·076 | 0·308 | 0·494 | 0·198 |
| Sunderland | 0·616 | 0·338 | 0·046 | 0·214 | 0·497 | 0·289 |
| *Greece* | | | | | | |
| Athens | 1·000 | 0·000 | 0·000 | 1·000 | 0·000 | 0·000 |
| Yanninan | 0·477 | 0·427 | 0·096 | 0·352 | 0·483 | 0·165 |
| *Hungary* | | | | | | |
| Budapest | 0·0001 | 0·0228 | 0·9771 | 0·003 | 0·104 | 0·893 |
| *Ireland* | | | | | | |
| Cork | 0·609 | 0·343 | 0·048 | 0·144 | 0·471 | 0·385 |
| Galway | 0·392 | 0·468 | 0·140 | 0·179 | 0·488 | 0·333 |
| Longford | 1·000 | 0·000 | 0·000 | 0·488 | 0·421 | 0·091 |
| *Israel* | | | | | | |
| Huleh District | 0·794 | 0·194 | 0·012 | 0·794 | 0·194 | 0·012 |

APPENDIX II—*continued*

| Localities | Glucoside genotypes | | | Enzyme genotypes | | |
|---|---|---|---|---|---|---|
| | $p^2$ | $2pq$ | $q^2$ | $p^2$ | $2pq$ | $q^2$ |
| | *AcAc* | *Acac* | *acac* | *LiLi* | *Lili* | *lili* |
| *Italy* | | | | | | |
| Lucca | 1·000 | 0·000 | 0·000 | 0·702 | 0·272 | 0·026 |
| Milan | 0·0004 | 0·0347 | 0·9649 | 0·008 | 0·167 | 0·825 |
| San Daniele del Friuli | 0·001 | 0·061 | 0·938 | 0·005 | 0·132 | 0·863 |
| *Jugoslavia* | | | | | | |
| Ljubljana | 0·005 | 0·128 | 0·867 | 0·004 | 0·122 | 0·874 |
| *Lebanon* | 1·000 | 0·000 | 0·000 | 1·000 | 0·000 | 0·000 |
| *Liechtenstein* | | | | | | |
| Schaanwald | 0·155 | 0·477 | 0·368 | 0·093 | 0·424 | 0·483 |
| *Netherlands* | | | | | | |
| Breda | 0·100 | 0·432 | 0·468 | 0·104 | 0·437 | 0·459 |
| Groningen | 0·009 | 0·169 | 0·822 | 0·003 | 0·094 | 0·903 |
| *Norway* | | | | | | |
| Tjøtta | 0·0009 | 0·0591 | 0·9400 | 0·0004 | 0·0396 | 0·9600 |
| *Poland* | | | | | | |
| Kościan | 0·0004 | 0·0396 | 0·9600 | 0·002 | 0·078 | 0·920 |
| *Spain* | | | | | | |
| La Coruña | 1·000 | 0·000 | 0·000 | 0·621 | 0·334 | 0·045 |
| Gerona | 1·000 | 0·000 | 0·000 | 0·421 | 0·456 | 0·123 |
| Granada | 1·000 | 0·000 | 0·000 | 1·000 | 0·000 | 0·000 |
| *Switzerland* | | | | | | |
| Lausanne | 0·390 | 0·469 | 0·141 | 0·325 | 0·490 | 0·185 |
| Zürich | 0·244 | 0·500 | 0·256 | 0·181 | 0·429 | 0·390 |
| *Turkey* | | | | | | |
| Adapazari | 1·000 | 0·000 | 0·000 | 0·287 | 0·498 | 0·215 |
| Samsun | 1·000 | 0·000 | 0·000 | 0·261 | 0·500 | 0·239 |
| Kayseri | 1·000 | 0·000 | 0·000 | 0·510 | 0·408 | 0·082 |

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# INDUCTION, PAR LE CHLORURE DE TÉTRAZOLIUM, DE LA MUTATION " PETITE COLONIE " CHEZ LA LEVURE

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## 1. INTRODUCTION

Selon Raut (1953) les mutants à déficience respiratoire de la levure (" petite colonie ", Ephrussi, Hottinguer et Chimenes, 1949) proliférant sur milieu solide additionné de sels de tétrazolium, forment des colonies blanches, alors que les colonies formées dans les mêmes conditions par une levure normale sont roses.

Au cours d'expériences destinées à mettre au point une technique de diagnostic différentiel des deux types de levure, fondée sur l'observation de Raut, Ephrussi (communication verbale) a observé que le chlorure de tétrazolium inhibe électivement la croissance des levures normales et augmente la proportion de colonies de taille réduite. Les caractéristiques biochimiques des cellules constituant ces " petites colonies " sont apparemment identiques à celles des mutants à déficience respiratoire dont la formation spontanée et l'induction par les acridines ont été décrites par Ephrussi et ses collaborateurs. L'augmentation de la proportion de mutants dans les populations proliférant en présence de tétrazolium pouvait, *a priori*, être due soit à une action sélective, soit à une action mutagène de cette substance. C'est afin d'en éclaircir le mode d'action que nous avons entrepris les expériences décrites ci-dessous.

## 2. MATERIEL ET TECHNIQUES

Toutes les expériences sont effectuées sur la levure de boulangerie (*Saccharomyces cerevisiæ*), souche B II (diploïde).

Les cultures en milieu liquide sont faites dans des fioles d'Erlenmayer contenant 25 $cm^3$ d'eau de touraillons (Ephrussi, Hottinguer et Chimènes, 1949) agitées à 25°. L'inoculum comprend environ 5000 cellules.

L'observation des colonies est faite à la suite d'étalements des cultures sur le milieu : Yeast extract, 0,5 p. cent. ; glucose, 3 p. cent. ; gélose, 2 p. cent., en boites Petri et incubation à 25°.

Le même milieu, dépourvu de gélose, est utilisé pour l'isolement des cellules de levure en goutte pendante dans la chambre humide de Winge, à l'aide du micromanipulateur de Fonbrune. La culture de ces cellules a lieu à 25° également.

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Le sel de tétrazolium (TZ) utilisé est le chlorure de 2.3.5. triphényltétrazolium fourni par Paul Lewis Laboratories, Milwaukee, Wisconsin,

$$R-C\begin{matrix}N-N-R' \\ \| \quad\quad Cl- \\ N-N^{+}-R'\end{matrix} \; ; \; R = R' = R'' = C_6H_5-$$

Une solution aqueuse à 1 p. cent. est stérilisée par filtration et conservée au froid et à l'obscurité. Au cours des expériences, les solutions sont exposées le moins longtemps possible à la lumière.

Les concentrations du TZ seront exprimées en gr. par litre.

## 3. RESULTATS ET CONCLUSIONS

Des suspensions de levures sont étalées sur milieu gélosé additionné de diverses quantités de TZ. Pour les concentrations les plus fortes ($1 \times 10^{-3}$ et $5 \times 10^{-4}$), on ne décèle à l'oeil nu aucune croissance,

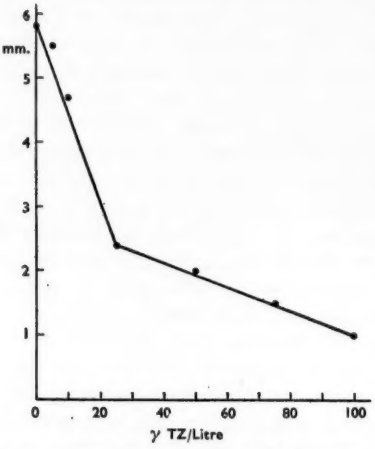


FIG. 1.—Diamètre des colonies (moyennes de 20 déterminations) en fonction de la concentration ($\gamma$/1.) du milieu en TZ.

même au bout de 5 jours d'incubation. Par contre, dans les cultures témoins, dépourvues de TZ, on observe des colonies au bout de 24 heures ; il en est de même lorsque la concentration du milieu en TZ est de $1 \times 10^{-6}$, $5 \times 10^{-6}$ ou $1 \times 10^{-5}$. Pour des concentrations en TZ de $5 \times 10^{-5}$ et $1 \times 10^{-4}$, les colonies apparaissent au bout de 48 à 72 heures.

Les différences dans la croissance en présence des différentes concentrations de TZ sont mises en évidence par la comparaison des diamètres moyens des colonies au bout de 4 jours d'incubation à 25°. Puisque la taille des colonies dépend du nombre de celles-ci par boite de Pétri, les mesures des diamètres sont faites dans des boites de Pétri ne contenant que 30 à 40 colonies.

Les résultats de ces mesures sont donnés par la courbe de la figure 1

dont le changement de pente à la concentration de $2{,}5 \times 10^{-5}$ indique le changement de la nature des colonies au delà et en deça de cette concentration de TZ. Les deux classes de colonies peuvent être différenciées par des tests plus rigoureux.

On sait que la réaction du Nadi, spécifique de l'indophénoloxydase, est négative chez les mutants " petite colonie " (Slonimski et Ephrussi, 1949) et positive chez la levure normale. Des échantillons de colonies ayant proliféré en présence de diverses concentrations en TZ sont prélevés et repiqués en milieu nutritif liquide. Après 10 passages de 48 heures les cellules sont soumises au test du Nadi. Les résultats obtenus sont les suivants : toutes les colonies prélevées sur les milieux contenant de $1 \times 10^{-4}$ à $2{,}5 \times 10^{-5}$ de TZ ont engendré des clones Nadi-négatifs. Par contre, tous les clones formés par des colonies prélevées sur des milieux à concentration en TZ inférieure à $1 \times 10^{-5}$ sont Nadi-positifs.

L'examen spectroscopique effectué sur les cultures dérivées des colonies ayant proliféré en présence de concentrations élevées en TZ ($1 \times 10^{-4}$ à $2{,}5 \times 10^{-5}$) met en évidence l'absence des bandes des cytochromes *a* et *b*, caractéristique des mutants " petite colonie " (Slonimski et Ephrussi, 1949). Ces bandes sont au contraire présentes chez les levures prélevées sur des milieux dépourvus de TZ ou n'en contenant que de faibles concentrations ($1 \times 10^{-6}$ à $1 \times 10^{-5}$).

En dehors des colonies normales observées sur les milieux contenant de 0 à $1 \times 10^{-5}$ de TZ, on y trouve une faible proportion de " petites colonie ", omises lors de la mesure des diamètres. Le tableau 1

TABLEAU 1

*Pourcentage de petites colonies formées en présence de diverses concentrations de TZ dans le milieu de culture*

| Concentration du TZ (gr./l.) | Nombre de colonies | | | Pourcentage des petites colonies |
|---|---|---|---|---|
| | Total | Normales | Petites | |
| $1 \times 10^{-4}$ | 2113 | ... | 2113 | 100 |
| $7{,}5 \times 10^{-5}$ | 2104 | ... | 2104 | 100 |
| $5 \times 10^{-5}$ | 2049 | ... | 2049 | 100 |
| $2{,}5 \times 10^{-5}$ | 2036 | ... | 2036 | 100 |
| $1 \times 10^{-5}$ | 2295 | 2249 | 46 | 2,0 |
| $5 \times 10^{-6}$ | 1993 | 1971 | 22 | 1,1 |
| $1 \times 10^{-6}$ | 660 | 652 | 8 | 1,2 |
| 0 | 2191 | 2172 | 19 | 0,9 |

donne la fréquence des colonies mutantes, après étalement d'échantillons d'une même suspension de levure sur des milieux contenant diverses concentrations de TZ.*

On voit que les pourcentages des petites colonies trouvés pour

* Seul l'étalement sur TZ à $1 \times 10^{-6}$ a été fait avec une suspension plus diluée.

les concentrations en TZ de $1 \times 10^{-6}$ et $5 \times 10^{-6}$ sont sensiblement égaux au pourcentage de mutants spontanés des cultures témoins. Un léger accroissement de ce pourcentage est observé sur TZ à $1 \times 10^{-5}$.* Enfin, toutes les colonies formées en présence de $2{,}5 \times 10^{-5}$ à $1 \times 10^{-4}$ de TZ sont du type " petite colonie ".

Le fait que le nombre total de colonies obtenues à partir d'ensemencements égaux reste sensiblement le même quelle que soit la concentration en TZ suggère que l'accroissement du pourcentage des " petites colonies " n'est pas dû à la destruction élective des cellules normales par le TZ.

Suivant la concentration de TZ dans le milieu gélosé, les colonies de levure sont plus ou moins fortement colorées. Lorsque la concentration du TZ est comprise entre $1 \times 10^{-4}$ et $2{,}5 \times 10^{-5}$, la plus grande partie des colonies sont rouge pâle à rose pâle ; quelques colonies sont blanches. Lorsque la concentration est comprise entre $1 \times 10^{-5}$ et $1 \times 10^{-6}$, les colonies normales sont foncé rouge ou roses, les petites colonies roses ou blanches.

Il ressort de ce qui vient d'être dit que, pour certaines concentrations du milieu de culture en TZ, il ne se forme que des colonies mutantes dont les cellules conservent leurs propriétés caractéristiques pendant de nombreuses générations.

Deux hypothèses peuvent rendre compte de ce fait :

(*a*) le TZ exerce un effet sélectif : dans un milieu contenant du TZ, les cellules mutées ont un avantage sélectif sur la forme normale. En conséquence, les mutants spontanés qui apparaissent dans toute culture de levure normale prennent le dessus en présence de TZ.

(*b*) le TZ a une action mutagène : il induit la mutation " petite colonie ".

Se fondant sur les principes établis par Luria et Delbrück (1943), Ephrussi, Hottinguer et Leupold (inédit) ont mis au point une technique qui permet de trancher entre les deux hypothèses et de comparer les taux de mutation dans deux séries de cultures, en l'occurrence en présence et en absence de TZ.

Une goutte d'une culture de levure est placée dans la chambre à microdissection. Des cellules individuelles y sont prélevées et placées isolément soit dans des gouttelettes de milieu normal (témoins), soit dans des gouttelettes du même milieu contenant $5 \times 10^{-5}$ de TZ. La chambre est incubée à 25°. Lorsque le nombre de cellules dans les clones formés par chacune des cellules isolées atteint 100 à 200, les cultures sont retirées de la chambre à microdissection par aspiration dans une micro-pipette et étalées sur milieu gélosé normal en boites

* A cette concentration, le pourcentage des petites colonies est encore faible, mais toutes les grandes colonies sont " festonnées ". D'apres Ephrussi et ses collaborateurs (Ephrussi, 1949 ; Ephrussi, L'Héritier et Hottinguer, 1949), une colonie festonnée, dissociée et réétalée, donne naissance à un mélange de grandes et de petites colonies. Les colonies festonnées sont donc probablement le résultat d'un équilibre entre cellules normales et cellules mutées.

de Pétri. Au bout de 3 à 4 jours de croissance à 25°, le nombre de colonies de chaque type (" grandes " et " petites ") est déterminé.

Le tableau 2 donne les résultats d'une expérience typique, et le tableau 3 un résumé des résultats de l'ensemble des expériences effectuées.

TABLEAU 2

*Fréquence des petites colonies dans les clones formés par des cellules isolées en milieu normal ou contenant du TZ (résultats d'une expérience type)*

| Rapport colonies/cellules | | Nombre de colonies | | | | | | Pourcentage des petites colonies | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total | | Normales | | Petites | | | |
| TZ | Témoin | TZ | Témoin | TZ | Témoin | TZ | Témoin | TZ | Témoin |
| 0,53 | *0,58* | 46 | *47* | 34 | *46* | 12 | *1* | **26,1** | *2,1* |
| 0,54 | *0,41* | 52 | *42* | 43 | *42* | 9 | *0* | **9,3** | *0* |
| 0,63 | *0,51* | 74 | *54* | 57 | *54* | 17 | *0* | **22,9** | *0* |
| 0,58 | *0,59* | 34 | *61* | 0 | *61* | 34 | *0* | **100** | *0* |
| 0,51 | *0,55* | 62 | *51* | 56 | *51* | 6 | *0* | **9,7** | *0* |
| 0,60 | *0,60* | 58 | *70* | 38 | *70* | 20 | *0* | **34,5** | *0* |
| 0,69 | *0,57* | 74 | *51* | 70 | *51* | 4 | *0* | **5,4** | *0* |
| 0,63 | *0,63* | 59 | *83* | 35 | *83* | 24 | *0* | **40,7** | *0* |
| 0,76 | *0,57* | 86 | *56* | 55 | *56* | 31 | *0* | **36,0** | *0* |
| 0,46 | *0,65* | 55 | *96* | 40 | *96* | 15 | *0* | **27,3** | *0* |
| 0,50 | *0,58* | 46 | *105* | 33 | *104* | 13 | *1* | **28,3** | *0,9* |

On constatera tout d'abord que le rapport entre le nombre de cellules dans un clone et le nombre de colonies formées après étalement de celui-ci sur milieu gélosé est le même (0,69-0,70) pour les clones ayant proliféré en présence et en absence de TZ. La valeur de ce

TABLEAU 3

*Résumé de l'ensemble des expériences sur la fréquence des petites colonies dans les clones formés par des cellules isolées en milieu normal contenant du TZ*

| Milieu | Nombre de | | | Rapport : colonies/ cellules | Nombre de colonies | | Pourcentage de petites colonies | Clones sans petites colonies |
|---|---|---|---|---|---|---|---|---|
| | clones | cellules | colonies | | normales | petites | | |
| TZ | 38 | 4634 | 3229 | 0,70 | 2450 | 779 | 24,1 | 2 |
| Normal | 43 | 6687 | 4612 | 0,69 | 4585 | 27 | 0,6 | 26 |

rapport s'explique de la façon suivante : lorsqu'on compte le nombre de cellules dans un clone, on compte forcément un certain nombre de bourgeons qui ne se détachent pas de la cellule-mère lors de l'étalement. Ainsi, dans un certain nombre de cas, ce qu'on a compté comme 2 cellules ne donne naissance qu'à une seule colonie.

On constate d'autre part que le pourcentage des petites colonies

est beaucoup plus élevé dans les clones ayant proliféré en présence de TZ (24,1 p. cent.) que dans les clones témoins (0,6 p. cent.), et que la proportion de clones n'ayant formé aucune petite colonie est beaucoup plus faible dans la série TZ que dans la série témoin (tableau 3, dernière colonne).

Ces chiffres permettent de calculer les taux de mutation pour les deux séries de cultures, selon les principes indiqués par Luria et Delbrück (1943). Afin d'éviter le facteur sélection, on calcule les taux de mutation *a* à partir du rapport : *clones sans mutants/nombre total de clones*, en utilisant l'équation :

$$a = \frac{ln2 \times ln(C_0/C)}{N}$$

où $C_0$ est le nombre de clones sans mutants, C le nombre total de clones, et N le nombre moyen de colonies par boite de Pétri. Ce calcul fournit $a = 2{,}3 \times 10^{-2}$ pour les cultures en TZ et $3{,}3 \times 10^{-3}$ pour les témoins, et montre donc qu'*à la concentration de* $5 \times 10^{-5}$ *le TZ cause une élévation du taux de mutation de* 10 *fois environ.*

Notons cependant que la différence entre les taux de mutation dans les deux séries de cultures est probablement sous-estimée par ce calcul. Celui-ci est en effet fondé sur les chiffres des tableaux 2 et 3 qui, on se souvient, sont le résultat du classement des colonies d'après leur diamètre. Or, si l'on prélève des colonies ainsi classées " petites " et si on les soumet à une série de passages, suivis du test de la réaction du Nadi, on trouve que la très grande majorité des clones dérivés des colonies de taille réduite de la série témoin sont Nadi-positifs, tandis que tous les clones correspondants de la série TZ sont Nadi-négatifs.

Des échantillons provenant de 14 petites colonies trouvées dans les cultures en TZ et de 14 petites colonies trouvées dans les cultures témoin ont été prélevés. Après 10 passages de 48 heures en milieu liquide dépourvu de TZ, les cultures ont été soumises à la réaction du Nadi. Cette réaction s'est révélée négative pour toutes les cultures de la série TZ et positive pour celles de la série témoin.

La nature des " fausses petites " (" petites réversibles " d'Ephrussi, L'Heritier et Hottinguer, 1949) étant inconnue, il serait raisonnable, du point de vue où nous nous plaçons dans ce travail, de ne considérer comme mutants véritables que ceux dans la descendance desquels on n'a pas observé de " réversion " vers la forme normale. Si l'on accepte cette manière de voir, il apparaît qu'en nous fondant sur le seul critère morphologique, nous avons, dans le calcul ci-dessus, considérablement surestimé la fréquence des mutations spontanées et sous-estimé la différence entre les taux de mutation dans les deux séries de clones, puisque les " fausses petites " sont particulièrement fréquentes dans la série témoin.*

* Le très petit nombre de déterminations ne nous permet de donner qu'une estimation *maxima* du taux de mutation spontané fondée sur l'emploi du test du Nadi. Elle fournit la valeur $1{,}1 \times 10^{-3}$. Selon Ephrussi et ses collaborateurs, la valeur réelle est plus proche de $1{,}1 \times 10^{-4}$ (communication personnelle).

Nous avons pensé qu'en utilisant des concentrations plus élevées de TZ il serait possible de donner une démonstration plus directe de l'action mutagène de cette substance par l'emploi de la technique d'Ephrussi et Hottinguer (1950). Une cellule (cellule-mère) provenant d'une culture normale de levure est placée à l'aide du micro-manipulateur dans une gouttelette de milieu nutritif contenant du

TABLEAU 4

*Résultats de l'étude de la descendance de 3 cellules proliférant en présence de TZ et de 3 cellules témoins*

| | TZ | | | Témoins | | |
|---|---|---|---|---|---|---|
| | Rapport : nombre des clones/cellules | Nombre de colonies | Pourcentage des petites colonies | Rapport : nombre des clones/cellules | Nombre de colonies | Pourcentage des petites colonies |
| $F_a$ | 0,53 | 61 | 100 | 0,67 | 189 | 0 |
| $F_b$ | 0,74 | 104 | 100 | 0,45 | 90 | 1,1 |
| $F_c$ | 0,79 | 103 | 100 | 0,56 | 70 | 0 |
| $F_d$ | 0,86 | 99 | 100 | 0,55 | 62 | 0 |
| $F_e$ | 0,94 | 254 | 92,1 | 0,69 | 83 | 1,2 |
| $F_f$ | 0,78 | 46 | 100 | 0,61 | 85 | 0 |
| $F_g$ | 0,80 | 104 | 100 | 0,62 | 124 | 0 |
| P | 0,71 | 59 | 72,2 | 0,79 | 126 | 0 |
| $F_a$ | 0,89 | 286 | 0,7 | 0,76 | 129 | 0 |
| $F_b$ | 0,68 | 93 | 100 | 0,96 | 173 | 0 |
| $F_c$ | 0,63 | 104 | 58,6 | 0,64 | 103 | 1 |
| $F_d$ | 0,77 | 93 | 100 | 0,93 | 109 | 0,9 |
| $F_e$ | 0,59 | 71 | 100 | 0,27 | 34 | 0 |
| $F_f$ | 0,53 | 64 | 26,6 | 0,55 | 68 | 0 |
| P | 0,66 | 83 | 38,5 | 0,86 | 216 | 0 |
| $F_a$ | 0,74 | 221 | 0,4 | 0,65 | 175 | 0 |
| $F_b$ | 0,57 | 102 | 76,5 | 0,61 | 104 | 0 |
| $F_c$ | 0,54 | 51 | 5,9 | 0,53 | 59 | 0 |
| $F_d$ | 0,66 | 83 | 100 | 0,55 | 70 | 0 |
| $F_e$ | 0,81 | 89 | 100 | 0,57 | 65 | 0 |
| $F_f$ | 0,84 | 77 | 100 | 0,67 | 61 | 0 |
| $F_g$ | 0,79 | 59 | 100 | 0,64 | 89 | 0 |
| $F_h$ | 0,88 | 106 | 100 | 0,56 | 57 | 1,7 |
| P | 0,68 | 71 | 100 | 0,68 | 61 | 1,6 |

TZ. Le bourgeonnement de la cellule-mère (P) est observé au microscope, et les cellules-filles ($F_a$, $F_b$, $F_c$, etc.) sont séparées de la cellule-mère au fur et à mesure de leur formation, et transportées chacune dans une gouttelette de milieu nutritif normal. A la fin de l'expérience, la cellule-mère elle-même est transportée dans une gouttelette de milieu normal. Des cellules isolées et traitées de la même façon dans du milieu dépourvu de TZ servent de témoin. Lorsque les cellules ainsi isolées ont formé des clones contenant de 100 à 200 cellules, ceux-ci sont retirés de la chambre à microdissection et étalés sur

milieu gélosé normal en boites de Pétri. Au bout de 3 à 4 jours, on y dénombre les colonies de taille normale et réduite, et on prélève un certain nombre des dernières pour les soumettre, après une série de repiquages, à l'examen spectroscopique.

Le tableau 4 donne, à titre d'exemple, les résultats de 6 expériences, dont 3 ont été effectuées en présence de TZ à $2 \times 10^{-4}$, 3 autres en milieu normal (témoins), et le tableau 5 résume l'ensemble des expériences réalisées avec cette concentration de TZ et des expériences témoins.

L'examen de ces tableaux permet de constater que

(1) Les clones témoins ne forment le plus souvent pas de petites colonies ou n'en forment qu'un petit pourcentage. Seulement dans 8 cas sur 48 le pourcentage des petites colonies a dépassé 2 per cent. (maximum observé : 22 per cent.).

TABLEAU 5

*Résumé des résultats fournis par l'étude de la descendance des cellules isolées en milieu normal ou contenant du TZ*

| Milieu | Nombre des cellules | | | Nombre de clones mixtes contenant plus de 2 p. cent. de " petites colonies " |
|---|---|---|---|---|
| | P | F | F mutées | |
| TZ | 7 | 45 | 18 | 17 |
| Normal | 6 | 48 | 0 | 8 |

(2) Par contre, parmi les colonies formées par les clones de la série TZ, un pourcentage élevé sont de taille réduite. Sur 45 clones, 17 ont formé plus de 2 per cent. de petites colonies et 18 autres ont formé uniquement des petites colonies, ce qui permet de conclure que les 18 cellules F qui sont à l'origine de ces clones étaient elles-mêmes mutées. Puisque aucun des 48 clones F de la série témoin ne s'est comporté de façon semblable, *il est clair que le TZ exerce une action mutagène*. Cette conclusion est encore confirmée par le fait que, dans la série TZ, les clones engendrés par les cellules-mères (P) après leur transfert dans le milieu normal ont formé soit uniquement des petites colonies, soit un mélange de colonies des deux tailles. Dans un certain nombre de cas, le TZ fait donc muter les cellules-mères elles-mêmes, ou y induit un " état instable " qui se manifeste par la production fréquente de bourgeons mutés (Ephrussi et Hottinguer, 1951). La mutation des cellules-mères au cours de l'expérience n'a jamais été observée chez les témoins, et les rares cas où les clones témoins contiennent un pourcentage relativement élevé de petites colonies doit être attribué à l'intervention précoce, dans l'histoire du clone, d'une mutation spontanée.

Les données fournies par les expériences qui viennent d'être décrites permettent de calculer les taux de mutation dans les séries TZ et témoins, suivant la formule

$$\pi = \frac{n_p}{n_g + n_p}$$

où $n_g$ est le nombre de grandes et $n_p$ le nombre de petites colonies formées par un certain nombre de cellules normales (Marcovich, 1951). Le calcul ne sera fondé, bien entendu, que sur celles des expériences en TZ où la cellule-mère elle-même n'a pas subi de mutation avant l'isolement du dernier bourgeon. D'autre part, on ne considèrera comme mutantes que les cellules-filles dont la descendance n'a formé que des petites colonies. Dans ces conditions, le calcul fournit, pour la série TZ, $\pi = 0{,}32$.* Puisque dans la série témoin aucune cellule F mutante n'a été trouvée, on ne peut donner qu'une estimation de la valeur maxima de $\pi$ qui est $<0{,}02$.

*Les expériences ci-dessus étant réalisées de façon à exclure le facteur de sélection (la mortalité des cellules y est pratiquement nulle), les résultats obtenus établissent l'induction, par le TZ, des mutants à déficience respiratoire.* On notera cependant qu'une analyse génétique serait nécessaire pour déterminer s'il s'agit ici de mutants cytoplasmiques ou géniques.

## 4. RÉSUMÉ

(1) En présence dans le milieu de culture de certaines concentrations de 2.3.5. triphényltétrazolium (TZ), les levures ne forment que des colonies de taille réduite qui présentent les mêmes caractéristiques morphologiques et physiologiques que les mutants à déficience respiratoire décrits par Ephrussi et ses collaborateurs.

(2) Le pouvoir mutagène du TZ a été établi par des expériences réalisées dans des chambres à microdissection, dans des conditions qui excluent la sélection, et les taux des mutations induites ont pu être calculés.

## 5. SUMMARY

(1) Baker's yeast, grown in the presence of certain concentrations of 2.3.5. triphenyltetrazolium chloride (TZ), produces dwarf colonies, the cells of which are morphologically and physiologically similar to the respiration deficient mutants described by Ephrussi *et al.*

(2) The mutagenic effect of TZ is established by experiments performed in the microdissection chamber, under conditions excluding selection. The induced mutation rate is determined.

Je tiens à remercier le Professeur B. Ephrussi de m'avoir suggéré le sujet de ce travail, ainsi que des conseils et des critiques qu'il a bien voulu me prodiguer.

* Ce calcul est fondé encore une fois sur les données fournies par l'observation des colonies. Le taux de mutation ainsi calculé doit cependant être très proche de la vérité puisque sur 32 petites colonies isolées dans ces expériences, 31 ont formé des clones dont l'examen spectroscopique, après 6 à 9 passages, a confirmé la nature mutante.

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# A MUTATION OR A CROSSOVER IN THE HOUSE MOUSE ?

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## 1. INTRODUCTION

DURING the last fifty years there have been several reports of genetic activity at the agouti locus in the house mouse, *Mus musculus*. The phenotypic equivalence of some genotypes involving $A^L$ (light-bellied agouti) and the genotype $Aa^t$ ($A$ is dark-bellied agouti and $a^t$ is black-and-tan) led Pincus (1929) to suggest that not one but two loci may be the genetic basis of the apparent multiple-allelism so far observed. On this theory, one locus governs the belly colour and the other that of the back; $W$* and $w$, representing white and not-white belly, and $A$ and $a$, representing agouti and non-agouti back, are the symbols used by Keeler (1931).

Professor Sir Ronald Fisher, F.R.S., suggests that $A^Y$ (yellow), because it differs from the other members of the series in several respects, *e.g.* in causing lethality when homozygous and in producing a tendency to obesity in heterozygotes, is due to a deletion covering more than one locus; some little support for this idea is given by the fact that none of the mutations so far reported have occurred in matings involving $A^Y$. This factor will not therefore be considered in this paper in the treatment of the evidence for one or two loci as a basis of the agouti series.

Until now the events described have been interpretable, on either theory, only in terms of mutation. The occurrence of a possible crossover in the stocks in this department here reported, seems a suitable occasion on which to survey and discuss the evidence available on the genetic basis of the agouti series.

## 2. MUTATIONS OBSERVED IN THE LABORATORY

The genetical circumstances in which mutations have been observed have been somewhat varied, and the evidence for their occurrence is not uniformly conclusive. A brief account will facilitate discussion.

Table I gives the mutations reported by the various authors to whom Gruneberg (1952) refers in a section on the agouti series; the case reported in this paper is added to the table (table 1).

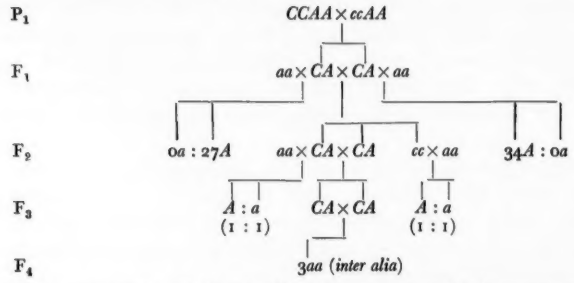
* $W$, $w$ used throughout this paper, does not refer to the locus at which dominant spotting occurs, which is normally given this symbol.

TABLE 1

*List of "mutations" reported at the Agouti locus*

| Year | Author | Number and genotypes of mutants | Parents of mutants |
|---|---|---|---|
| 1908, 1912 | Hagedoorn, A. L. | 2 *Aa* (in the same litter) | *AA*×*AA* |
| 1916 | Little, C. C. | 3 $A^L$ (each from a different monogamous mating) | *Aa*×*Aa* |
| | | 1 $A^L$ | *Aa*×*aa* |
| 1929 | Pincus, G. | 1 $a^t$ | *aa*×*aa* |
| 1931 | Snell, G. D. | 1 $a^t$, 1 $a^t$, 2 $a^t$ (each from a different monogamous mating) | *aa*×*aa*, *Aa*×*aa*, *Aa*×*Aa* |
| 1931 | Keeler, C. E. | 1 $A^L$ | *Aa*×$a^ta$ |
| 1947 | Little, C. C., and Hummel, K. P. | 3 $A^L$ (in different litters of the same mating) | *aa*×*aa* |
| 1949 | Bhat, N. R. | 1 $A^L$ | *aa*×*aa* |
| 1953 | Wallace, M. E. (reporting here) | 1 *aa* | $Aa^t$×*aa* |

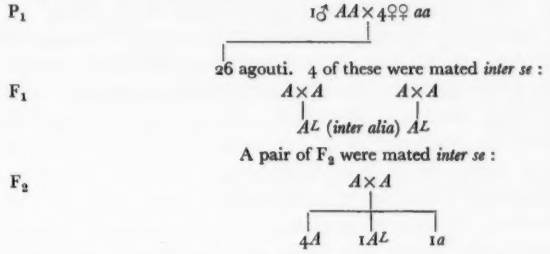
Hagedoorn's 1912 report is an expansion of his earlier paper. His notation is different from that in current use and rather unwieldy, and he mentions contemporary misinterpretation. It is therefore with some diffidence that a simplified account is given here, although the material described is not complicated. In current notation, the phenotypic genealogy can be summarised :




The genotype of both the $F_1$ mates, which were ancestors of the three black non-agoutis (*aa*), was proved to be *AA*, since they gave, in a testcross to non-agouti, 34 and 27 young each, all agouti. The descendants of the $F_1$ mates were also tested by crosses to non-agouti and one of the $F_2$ agoutis and the albino (*cc*) were found to be *Aa*. Hagedoorn gives no figures from the latter testcrosses, merely stating that the heterozygotes gave "equal numbers" of agoutis and non-agoutis, and that "the numbers of young produced from each test-mating always exceeded eight". He concludes from these data that "this heterozygous nature of the two young produced from homozygous parents can only be explained by assuming that one of the parents had produced at least two gametes without *G*" (*G* is his symbol

for current *A*). Since the two heterozygotes are stated to be from the same litter, it is possible that, if one parent produced two *a* gametes, the *a* genes could have arisen from the same or from separate mutations before or during gametogenesis, or that the parent was a gonadial mosaic. It is also, of course, possible that both parents were involved, producing one *a* gamete each. However, it is not easy to discriminate which explanation is the most likely, and it is sufficient for the purpose of this paper to show that the evidence for the occurrence of one, if not two, mutations *A* to *a* is strong.

Little (1916) describes clearly a large experiment, from which the relevant genealogy is here summarised :




A fourth mutation to $A^L$ occurred in a different experiment from a mating $Aa \times aa$. That three of the mutants were closely related is clear since they all arose from the same stock within three generations of inbreeding. Tests were made to establish (i) the allelism of the mutants (two of the mutants " have shown that their colour pattern was epistatic to grey-bellied agouti * and non-agouti "), and (ii) the identity with $A^L$ of the fourth mutant which had a rather yellow belly. Little also states that the possibility of a mistaken identity

" is obviated by the fact that the $F_2$ generation white-bellied agoutis appeared in different cages at the Harvard Medical School where there had been no other white-bellied agoutis † for more than a year before the appearance of the mutant animals ".

Reassurance is thus given for the conclusion that four mutations to $A^L$ occurred in Little's stock ; but it is not possible to distinguish whether the mutating genes were *A* or *a*.

Pincus' 1929 paper is a conclusive account of the occurrence of a mutation *a* to $a^t$. A black-and-tan female, " No. 7048 " appeared

" in the ninth brother-sister generation of an inbred line of chinchilla non-agouti (black) piebald mice. . . . That this animal represented a mutation was apparent for the following reasons : (1) Its parents . . . produced in all 14 young in four litters, only one of which . . . showed the ventral coloration described ; (2) ♀7048, bred to totally unrelated non-agouti dark-bellied males, produced about equal numbers of white-bellied and dark-bellied young (20 light-bellied black and 10 dark-bellied black) ; (3) three of her sisters back-crossed to her father

* Grey-bellied agouti is *A*.

† White-bellied agouti is $A^L$

produced 16 young all dark-bellied ; (4) a brother and sister mated together produced 9 young, all dark-bellied. From these facts alone it will be seen that white belly represents a dominant mutation in one of the parental chromosomes so that a single animal heterozygous for the mutant factor appeared."

Pincus also satisfactorily eliminates the possibility of mistaken identities :

" First, no black and tan animals were in the laboratory at the time. . . . Second, the mutant resembled her litter mates in every respect."

Finally, he establishes the identity of his " white-bellies " with the black-and-tan described by Dunn (1928).

Snell (1931) reports the occurrence of four " probable mutations " to $a^t$, all born within three months of each other. The mutating gene in one case was certainly *a* since the parents were both *aa*, and in the other cases they may have been *A* or *a* : the parents of one were *Aa* and *aa*, and of the other two, both parents were *Aa*. Unfortunately two of the mutants died before breeding, and from the other two no breeding tests are reported. No indication is given of the relationship of the mutants, so that it is not possible to consider the independence or otherwise of these mutations. There is, however, some evidence, although necessarily inconclusive, that none of the mutants was in reality a black-and-tan transposed from other matings involving black-and-tans which did exist in the laboratory ; the genotypes of two of the mutants was in accord with expectation from their supposed parents. The four cases reported here can only be regarded, as Snell states, " as being, rather more probably than not, mutations to black and tan."

Keeler (1931) reports an unusual case. It can be summarised as follows :

$P_1$ $A \times a^t$

$F_1$ Several " *Aat* " were crossed to *aa* and one such mating gave :

$F_2$ 13 *aa* 19 $A^L$

The paper does not state explicitly, but implies that the mates of the $P_1$ cross could have been *Aa* and $a^t a$. Keeler mentions the improbability of a crossover occurring in the zygote, " an unheard-of occurrence ", and concludes

" Hence it seems probable that a mutation from *w* to *W* occurred in the parental gamete furnished by the agouti parent, or what seems less likely a reverse mutation from *a* to *A* in the parental gamete furnished by the black-and-tan parent."

He does not consider the single-locus theory, on which the mutating genes may have been *A*, $a^t$ or *a*. On the theory of two loci, the genealogy can be represented :

$P_1$ $\frac{Aw}{aw} \times \frac{aW}{aw}$

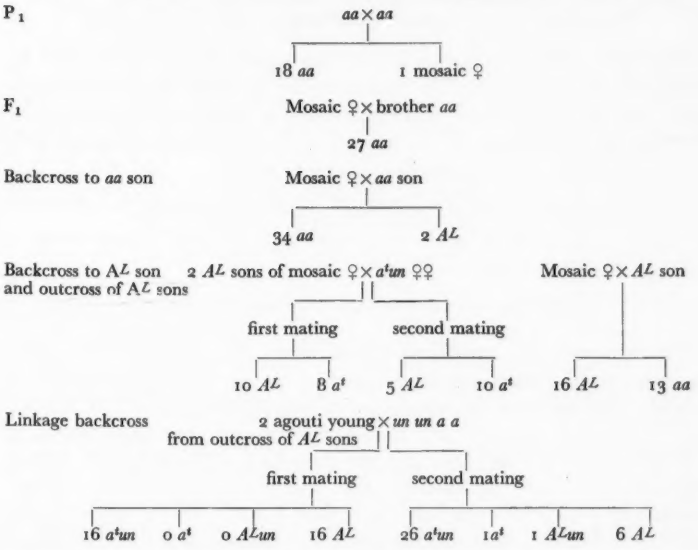
$F_1$ $\frac{AW}{aw}$

Little's (1947) report is the first case in which gonadial mosaicism is clearly indicated.

" The occurrence of three $A^W a$ * individuals in different litters from a single mating of the closely inbred dilute brown (*dba*) strain of mice, which has previously produced only *aa* animals since 1909, has been observed and is here recorded. The three individuals occurred among the progeny of ♀1 and ♂10, there being 18 normal young. . . . The frequency of the appearance of the mutant mice in the original mating suggests that one gonad of the mutating parent is a mosaic in respect to the formation of $A^W$ and *a* gametes."

The mutation's frequency of appearance

" is sufficient to suggest a very early division of the gonad of the mutating parent into ' $A^W a$ ' and ' *aa* ' bearing cells."

No breeding tests of the $A^W$ animals are reported. The possibility of an alternative explanation of the appearance of three closely related phenotypically identical mutants is, however, considered by

Little. He suggests that both parents producing the mutants were $+m$, producing $\frac{1}{4}mm$ which causes a mutation *a* to $A^W$ in one of the pairs of chromosome V. This explanation is considered by him to be " somewhat improbable ". He states that an investigation is in progress ; but a private communication reports that an accident destroyed the material before any further progress was made.

Bhat's (1949) case is unique for this locus in that the mutant mouse was a mosaic somatically as well as gonadially ; thus the origin of

* $A^W$ is alternative to the symbol $A^L$ used throughout this paper.

the mutation is more certainly known than in most cases. He describes the appearance from non-agouti parents of " an altogether new type of animal possessed of irregular agouti-like markings on the back . . . an agout-non-agouti mosaic." The possibility of its arising from an agouti parent is eliminated on genetical grounds, and the allelism with the agouti series of the light-bellied agouti gametes it produced is established by a linkage test with *un* (undulated). Bhat concludes that " in all probability it was a reversion of the gene *a* to $A^L$ or an equivalent allele." He does not exclude the possibility of its being " a mimic of $A^L$ but closely linked to it," but continues that this " though not inadmissible, is too far fetched to be of any special significance." The breeding tests described by Bhat are summarised below : some additional data available in this Department have been added.

## 3. A POSSIBLE CROSSOVER (OBSERVED IN THE LABORATORY)

The first evidence of activity at the agouti locus which can be interpreted as a crossover (as well as on a mutational basis) is now given. In the stocks in this Department, a mating E692 gave the following performance :

$$\text{♀}\ \frac{+}{py}\,\frac{B}{b}\,\frac{Sd\ fi\ a^t}{+\ +\ A} \times \text{♂}\ \frac{py}{py}\,\frac{B}{b}\,\frac{+\ fi\ a}{+\ fi\ a}$$

35 *A* 1 ♀ $\frac{py}{py}$ *B* $\frac{+\ fi\ a}{+\ fi\ a}$ 29 $a^t$

(*Sd* is Danforth's short tail, *fi* is fidget and *b* is brown.)

Unfortunately the single *aa* mouse from this mating died while pregnant by an unrelated non-agouti male, a mating intended to establish allelism with the agouti series and identity of *aa*. That her mother was $Aa^t$ and not $A^La$ is evident from (i) her production of 35 *A* and 29 $a^t$ young, and (ii) the fact that a dark-bellied agouti daughter and son produced, by unrelated non-agouti mates, respectively 3 *A* and 2 *aa*, and 4 *A* and 2 *aa*. The *A* gene of the mother was derived, *via* a mating $a^ta \times Aa$, direct from a stock in which there had never been any $A^L$.

It is very unlikely that the *aa* mouse was accidentally transposed from another mating, for the following reasons. Firstly, the *aa* mouse was also *fifi pypy B*, a genotype expected (excluding consideration of the agouti locus) with a fair frequency, about 1 in 20. Secondly, although there were other matings close by producing this genotype, this female appeared to be of the same age as her supposed litter-mates when the litter was classified for agouti, fidget and black, at 18 days old ; and the number and genotypes of the young in the cage then agreed with the number and genotypes observed when the litter was

first recorded at two days old and classified for sex, *Sd* and polydactyly. The very varied expression of polydactyly is fully described in these stocks, and it would be unusual to find many animals of the same age, sex and *Sd* genotype at any one time with the same degree of expression of this factor. Moreover, other factors, including *W*, *p* and *d* (dominant pied, pink-eyed dilution and Maltese dilution), not segregating in this mating, were segregating in most of the other matings in the stock.

On this evidence, it seems likely (i) that a mutation to *a* occurred from *A* or $a^t$, or (ii) that a crossover *w*/*a* occurred. This can be represented (using Keeler's notation), as follows :

$$\frac{Aw}{aW} \times \frac{aw}{aw}$$
$$\Big|$$
$$\frac{aw}{aw}$$

## 4. MUTATIONS OBSERVED IN WILD POPULATIONS

There have been several reports of white-belly, made from observations on trapped wild specimens. The evidence on the genetic basis of the white-belly is not uniformly clear, and a short résumé would perhaps be useful.

TABLE 2

*List of reports of white-bellied agouti observed in the wild*

| Year | Author | Location of wild population |
|---|---|---|
| 1908 | Morgan, T. H. | Massachusetts |
| 1912 | Dice, L. R. | California |
| 1914 | Clarke, W. E. | St Kilda Island, Scotland |
| 1936 | Elton, Ch. | Coalmine in Ayrshire, Scotland |
| 1938 | Philip, U. | |
| 1946 | Eaton, O. N., and Schwarz, E. | Virginia |
| 1947 | Falconer, D. S. | |
| 1948 | Engels, W. L. | Some North Carolina coastal islands |
| 1949 | Zimmermann, K. | Middle Europe |

Morgan's (1908) report is very brief and gives no figures : " When the sport with a white belly is crossed with domesticated spotted mice the white-bellied character dominates." There is no indication of any breeding other than this initial outcrossing.

Dice (1912) is not concerned with the genetic basis of variation, but only with variation itself : accordingly, his account mentions no breeding tests at all :

" A considerable number of house mice in California have the under parts separated in color from the upper parts. The upper parts retain the color of the common house mice of the region, while the under parts become colored either white, creamy buff, reddish buff, or intermediate tints between these colors

and the color of the underparts of the unmodified house mouse. In all, seven house mice with white underparts, two with creamy buff underparts and a much larger number with reddish buff under parts have been taken. . . . Enough intermediate stages between the various colors have been found so that it becomes certain that these grade into one another and therefore are probably the product of the same factor or factors of variation."

Engels (1948) referring to Clarke (1914) states "The mice of St Kilda Island were found to be exclusively white-bellied agouti, and hence presumably all homozygotes." Clarke's paper is purely descriptive, and uses no genetic terms or symbols.

Elton (1936) describes a colony of mice found in a coalmine in Ayrshire. Philip (1938) describes breeding experiments with 12 specimens, of which 6 were received directly from Elton and the others were taken from a colliery connected with the pit from which Elton took his mice. Six of the mice were yellow-bellied and

"when crossed with grey-bellied agoutis of the pure line (Strong CBA) gave only yellow-bellied offspring. Yellow-belly is thus shown to be a dominant and to have been present in homozygous form in the animals obtained. The backcross of the $F_1$ to the homozygous grey-bellied agouti gave 18 yellow-bellied and 21 grey-bellied offspring, showing that the difference was due to a single pair of allelomorphs. . . . Each animal was tested by 10; 12; 12; 13; 14; 16 offspring respectively."

—these numbers presumably refer to the six $F_1$ progenies from the six outcrosses. The wild dark-bellied mice were crossed with lighter agouti tame mice, and the outcrosses, and backcrosses and intercrosses therefrom gave various shades not easily assigned to a definite number of classes. The absence of heterozygotes for grey- and yellow-belly in her sample leads Philip to conclude that the mice in the coal pit "were not mating at random but break up into comparatively small breeding units."

Falconer (1947) shows that the "snowy-belly" found by Eaton and Schwartz (1946) in Virginia was probably not a new allele but an expression of $A^L$ in the presence of modifiers. He establishes the identity of $A^L$ by breeding tests.

Engels (1948) mentions only "six wild-caught white-bellied mice successfully tested", from a total of thirty trapped. They were bred to wild grey-bellied mice, and apparently no laboratory animals were used at all. One white-bellied mouse produced no grey-bellies out of 20 offspring; "six white-bellied mice"—it is not obvious whether these include the one just mentioned—"each with a grey-bellied mate, produced . . . 28 white-bellied and 19 grey-bellied. . . . Chi-square for the observed deviation from the 1 : 1 ratio is 1·72, a sufficiently satisfactory fit." There were, therefore, one homozygote and five (or six?) heterozygotes, white-belly being thus proved dominant to grey-belly.

"Further evidence of the dominance of white-belly is afforded by two white-belly by white-belly matings involving one wild-caught mouse and three cage-bred mice of white-belly by grey-belly parentage."

These gave 9 white-bellies and 6 grey-bellies :

" Chi-square for the observed deviation from the expected 3 : 1 ratio is 1·80, a sufficiently satisfactory fit."

Engels concludes

" The above evidence identifies the gene involved as $A^W$, the dominant ' white-bellied agouti ' allelomorph of ' agouti ' . . ."

Zimmermann's 1949 paper is a survey of the distribution of various sub-species and variants of house mice. As such, it mentions no genetic tests, although it uses genetic symbols as if there had been genetic evidence for their use :

" Ein weiteres Kennzeichen von *domesticus* ist das Fehlen einer Demarkationslinie an den Flanken. Diese Färbung wird in der Genetik als ' Agouti ' (Symbol *A*) bezeichnet. Die ' Wildfärbung ' von *musculus* und *spicilegus*, bei der eine mehr oder weniger deutliche Trennung zwischen Oberseiten- und Bauchfärbung besteht, wird genetisch als ' Agouti weissbäuchig ' (Symbol $A^W$) bezeichnet, $A^W$ ist dominant über *A*."

And later :

" Gelbes Pigment in den Distalhälften der Bauchaare tritt auch in Verbindung mit der A-Farbung bei *domesticus* auf. Bei deutschen *musculus* überwiegen die hellen Bauchfärbungen."

Some indication that $A^W$ is in fact present in the feral sub-species is given by a mention of " snowy-belly " :

" Innerhalb der Wildfärbung bestehen für die Pigmentierung der Bauchhaare starke Schwankungen. Die Bauchhaare können völlig pigmentfrei sein und schneeweiss erscheinen (' snowy belly ', $A^s$), nach Eaton & Schwarz . . . ein weiteres Allel der Agouti-Serie, dominant über *AW*. ' Schneeweisser Bauch ' ist vorherrschend bei Hausmäsuen südlicher Trockengebiete, bei *musculus* und *spicilegus* tritt er nur als seltene Einzelmutante auf. (Im untersuchten Material 3 mal in Ostpreussen, 1 mal in Brandenburg, 1 mal unter 270 ungarischen *spicelegus*)."

Falconer (1947) suggests that, if in a population of *AA* with naturally selected modifiers for light bellies,

" a mutation from *A* to $A^W$ took place, the major effect of this allele in combination with the minor lightening genes might well produce the extremely light phenotype seen in the snowy-bellied mouse."

The specimens investigated by Falconer certainly carried $A^L$ : possibly, and for the same reason, those reported by Zimmermann also carried $A^L$. There cannot, however, be any certainty on this point, nor that the other " Agouti-weissbauchig " mice reported by Zimmermann also carried it.

## 5. DISCUSSION

Several cases of multiple-allelism have, of recent years, shown themselves to be, more probably, cases of close linkage—the Rhesus factor in Man being the classic example. An analysis of the evidence for these two genetic situations in a particular species is therefore of some value, and it is hoped that some of the arguments developed here may be of use in similar cases in other species.

In order to arrive at some conclusion, however tentative, as to the number and kinds of activity that have occurred at the agouti locus, it is necessary to examine the evidence and to reduce it to a small core about which there is some degree of certainty.

In a survey of this kind, each case must be judged on the material reported. Inevitably, some omissions and ambiguities in the reports must be pointed out, and possible explanations, other than those given by the authors, must be stated whatever their implications. It must be emphasised that the primary aim of this paper is not a criticism of technique, but an evaluation of the evidence reported.

First, a routine point which, if elaborated by the author, strengthens the claim for mutation, is that of identity of the anomalous animal. Not all the reports give assurance in this respect. For example, evidence in Keeler's (1931) paper against the possibility of mistaken identity would have been welcome—perhaps more so in this case than in others. For here the anomalous mouse was of the same phenotype as its supposed litter-mates : only its breeding behaviour distinguished it. It is unfortunate that no evidence from other segregating factors was available to give assurance as to its parentage. However, the fact that Keeler does not mention $A^L$ as present in his laboratory may perhaps be taken as circumstantial evidence in favour of mutation.

It would seem to be of importance that, in many cases, breeding tests were not made with the mutants. Their resemblance to a member of the agouti series is often taken as an indication of their identity with that member. Proof of identity has, empirically, three parts : the establishment of (i) heritability of the factor, (ii) identification of its locus, usually by allelomorphism with another factor at the locus, or by linkage with a neighbouring factor, and (iii) identity with a particular allele.

In cases where the expression of a gene is very variable, identity tests are particularly desirable. There is much published evidence of the variability of members of the agouti series, particularly as regards the belly colour (Little, 1916 ; Pincus, 1929 ; Schwarz and Schwarz, 1943 ; Kaliss, 1942 ; Falconer, 1947 ; Zimmermann, 1949 ; etc.). Thus, some of the reports of $a^t$ and $A^L$, occurring both in the laboratory and in the wild, omit description of identity tests or describe crosses made for other purposes, and they thus leave open the possibility of a polygenic explanation of the observations made. The examples whch follow are largely taken from reports of mutations in the wild where no knowledge of multi-factorial genotype is available until breeding tests are made, a circumstance which does not prevail to the same extent in the laboratory.

In Engel's (1948) reference to Clarke (1914), the word " presumably " seems to imply that genetic tests were not done to test the suggestion that all the mice of St Kilda were $A^LA^L$, and, in fact, Clarke's report does not mention breeding tests, being concerned

merely with descriptions of specimens. An advantage for white belly on this island may equally have resulted in a natural selection for factors lightening the expression of *AA*. From Dice's (1912) report there is little alternative to the conclusion that the variation he observed was genetically multi-factorial. Yet Gruneberg (1952) includes references to Clarke and Dice in a context which rather easily allows the interpretation that theirs are reports of mutations to $A^L$ :

" That mutations from $A$ to $A^W$ are possible follows from the occurrence of sporadic light-bellied mice in dark-bellied populations (*e.g.* cases reported by Morgan, 1908 ; Dice, 1912 ; Elton, 1936 ; Philip, 1938 ; Engels, 1948 ; Zimmermann, 1949). . . . It thus seems that mutations at the agouti locus are common both in the wild and in the laboratory. . . . There are even whole populations, such as that of the island of St Kilda (Clarke, 1914) in which $A^W$ has completely replaced $A^+$."

A further statement by the same authority is somewhat misleading :

" According to Schwarz and Schwarz (1943), all feral sub-species of *M. musculus* are $A^W$, and the dark belly of $A^+$ is the hall-mark of commensalism. However that may be, it is clear that $A^W$ and $A^+$ can be regarded as wild-type alleles of this species with equal justification."

Schwarz and Schwarz do not mention any breeding tests, but they do state :

" Specialisation in all commensals proceeds along the same lines. The wild forms are white-bellied and short-tailed, the most specialised commensals grey-bellied and long-tailed. There are intermediate types corresponding to the stage of commensalism."

It is difficult to make any certain conclusion from this, or from Zimmermann's paper, as to the incidence of $A^L$ in the wild. These two papers, and those of Dice and Clarke, do, however, indicate that some environments favour a light-belly, and one can conjecture —but it is no more than a conjecture—that mutation to $A^L$ could easily spread and even become homozygous in some populations. Falconer's (1947) suggestion about the origin of " snowy-belly " and Zimmermann's account of several snowy-belly mice caught in the wild, are some support to the view that mutations to $A^L$ are common in the wild ; but it appears that no certain conclusion can be drawn until more genetic tests have been made with wild specimens. On the present evidence, considering only those cases in which such tests have been described, there appear to be only three distinct mutations reported—Philip's, 1938 ; Engel's, 1948 ; and Falconer's 1947.

A further point on which fuller information would in some cases have been useful, is the relationship of apparently distinct mutations occurring at about the same time in the same laboratory. In Little's (1947) case, it is clear from the account that only one event affecting the agouti locus is necessary to account for the appearance of three " mutants ", *viz.* gonadial mosaicism in one parent. In Hagedoorn's (1908) and Little's (1916) reports, close relationship is described, a

circumstance which suggests the possibility of some genetic cause (other than mosaicism, at least in Little's case) for what has otherwise to be regarded as a very rare coincidence. Little satisfies himself that the recurrence of mutations to $A^L$ were not due to "inbreeding, hybridisation, selection." He describes a second inbreeding experiment parallel with the first, from which the mutants were obtained, and states that after three generations and the scoring of 4500 mice, "none of these animals were white-bellied agoutis." This experiment disposed of explanations which were possibilities according to contemporary knowledge; but it does not exclude the presence of a factor in the first experiment, absent from the second, which increases the mutation-rate at the agouti locus. Doubtless other explanations could be conceived if further evidence were available. That no details about relationship are given in Snell's (1931) account, is unfortunate since he reports the greatest number (four) of simultaneous mutations, all to the same allelomorph.

Although the phenotypic equivalence of $A^L$ and $Aa^t$ has been known at least since 1929 (Pincus), the necessity for establishing the identity of the factor causing light-belly in agoutis has not always been realised. Intercrosses of light-bellied animals (Engel, 1948) or crosses involving $a$ (Little, 1916) were apparently an unconscious safeguard in some cases; the fact that black-and-tan is not reported as appearing from such crosses is some reassurance that the mutations were in fact to $A^L$ and not to $a^t$. (It should perhaps be pointed out, however, that although from Engel's intercross 9 white-bellies to 6 grey-bellies is not a bad 3 : 1, this ratio is not a much worse approximation to the 1 grey-belly : 2 white-bellies : 1 black-and-tan expected if his cross was $Aa^t \times Aa^t$ and not $A^LA \times A^LA$.) No mention of the possibility of a mutation to $a^t$ is made in any of the reports of light-bellied agouti observed in the wild. In some cases, as for instance the evidence for homozygosity of light-belly in Philip's (1938) and in Engel's (1948) reports, there are in fact good grounds for believing that the mutating genes were in fact $A^L$ and not $a^t$. In general, however, the absence of evidence for the presence of $a^t$ rather than a definite report of the absence of $a^t$ has to be taken as indicating mutations to $A^L$ in the wild. Mutations to $a^t$, if they occurred, while producing light-bellies, would not become as widespread in wild stocks as those to $A^L$, since only the heterozygotes would have the advantageous belly colour. But it needs to be borne in mind, when studying wild populations, that sporadic mutations to $a^t$ may be picked up, and that there may even be balanced populations of three genotypes with $Aa^t$ the most common.

A final comment on the evidence must be made. It is striking that in all the reports of multiple mutations the mutations are always to the same gene. This seems an unusual coincidence, but it is doubtful whether there is any satisfactory explanation. Possibly it comes within human error: the observer, having noticed one mutation, tends to

interpret rather uncritically as similar mutations other anomalies which may have simpler explanations such as mistaken identity, multifactorial variation, etc. This argument reduces the estimate of the number of real mutations. On the other hand, there may have occurred more mutations than have been reported, but the observer, having seen one, tends to notice only similar rather than dissimilar ones. As it is impossible to discover the correct explanation, a conservative estimate of the number of real mutations will be made on the assumption that some human error probably has occurred, and that it has occurred more often in a direction which increases the apparent number than in the opposite direction.

To sum up: possible explanations of the unexpectedly large number of simultaneous mutations are as follows. Firstly, non-genetic causes:—mistaken identities; mistaken interpretation of observation; selective observation. Further, there may be some environmental agency, which stimulates a particular gene to mutate in a particular direction. This latter cause is not generally accepted, but it seems to have some reasonableness from the present evidence. Secondly, genetic causes: gonadial mosaicism (somatic or not); and a factor or factors increasing the mutation rate at the agouti locus.

With these possibilities in mind, and with the reservations indicated in the preceding paragraphs, some idea of the minimum number of events at the agouti locus may now be obtained (table 3).

Even when reduced to a minimum, the number of mutations to light-belly (whether or not the dorsal colouration is agouti or non-agouti) far exceeds the number of any other kind of mutation observed in the laboratory or in the wild.

Mutations to light-belly are far easier to observe in the wild than are those to dark-belly, since a greater number of populations of dark-bellies than of light-bellies are known; also, mutations to non-agouti (*a* on both theories) are difficult to observe because they are not distinct from the "wild" phenotype unless homozygous. But it is doubtful whether these considerations can appreciably alter the excess of mutations involving light-belly observed in the laboratory. Some degree of inbreeding is practised in a great number of experiments, and this process should reveal recessive mutations in stocks homozygous for any member of the series except *a*. Moreover, no mutation to dark-bellied agouti has been reported despite the fact that a large number of stocks are kept for the study or preservation of coat-colour mutants, and are run with members of the agouti series which are not agouti dorsally; whereas at least two mutations to light-bellied agouti have been reported as arising from stocks homozygous for non-agouti, in which mutations to dark-bellied agouti would be equally striking.

The real mutation-rate of each member of the series, and a final decision as to whether it is based on multiple allelism or close linkage,

cannot be obtained without a very large-scale experiment; such an experiment is not likely to be made, except perhaps incidentally in stocks conserving compatible factors, since the solution of this problem is at present not worth the labour this would require. At present, therefore, conclusions have to be drawn from data which may have a slight bias in favour of one mutation rather than of another. However, as there is no *a priori* reason for thinking the data biased, a tentative conclusion can be made that light-belly is more stable than dark.

TABLE 3

*A conservative estimate of the number of mutations at the agouti locus*

| Year | Author | Number and identity of mutating genes on basis of | |
|---|---|---|---|
| | | One locus | Two loci |
| | | A. *Observed in the laboratory* | |
| 1908 } 1912 } | Hagedoorn | 1 $A$ to $a$ | 1 $A$ to $a$ |
| 1916 | Little | 1 $A$ or $a$ to $A^L$ | 1 $w$ to $W \pm a$ to $A$ |
| 1929 | Pincus | 1 $a$ to $a^t$ | 1 $w$ to $W$ |
| 1931 | Snell | 1 $A$ or $a$ to $a^t$ | 1 $w$ to $W \pm A$ to $a$ |
| 1931 | Keeler | 1 $A$, $a$ or $a^t$ to $A^L$ | 1 $w$ to $W$ or $a$ to $A$ |
| 1947 | Little | 1 $a$ to $A^L$ | 1 $w$ to $W+a$ to $A$ |
| 1949 | Bhat | 1 $a$ to $A^L$ | 1 $w$ to $W+a$ to $A$ |
| 1953 | Wallace (this paper) | 1 $A$ or $a^t$ to $a$ | 1 $W$ to $w$ or $A$ to $a$ or a crossover in $\frac{Aw}{aW}$ giving $aw$ |
| | | B. *Observed in the wild* | |
| 1938 | Philip | $A$ to $A^L$ | $w$ to $W$ (in a coalmine in Ayrshire) |
| 1948 | Engels | $A$ to $A^L$ | $w$ to $W$ (some N. Caroline coastal islands) |
| 1947 | Falconer | $A$ to $A^L$ | $w$ to $W$ (via Eaton and Schwarz from Virginia) |

If this is so, and if there is only one locus, two alleles ($A^L$ and $a^t$) must be more stable than the other two. Whereas, if there are two loci, theory requires only one allele ($W$) to be more stable than its single counterpart. If it is admitted that "top-stability" is more likely to be confined to one rather than to two alleles, the theory of two loci has slightly more probability.

Incidentally, on the data there is no reason to suppose that dorsal agouti is more stable than non-agouti, although in general there have been slightly more mutations in this than in the opposite direction. If it is conceded on the theory of two loci that the loci are so close that a mutating agent affecting one would frequently affect the other simultaneously, then the two observed mutations $a$ to $A^L$ agree well with this theory. On the other hand, if there is only one locus, they

must be considered as mutations involving " two steps up at once " (*a*, via one of the two dominance-equivalents, *A* and $a^t$, to $A^L$), an event which future data may reveal to be more rare than simultaneous mutations of adjacent loci. To resume, on the basis of stability of light-belly, then, the most likely explanation appears to be that there are two loci, and that the allele *W*, and perhaps also the allele *A*, show relatively high stability.

In every case except the first a mutation " down " the series, that is, in the direction dominant to recessive, is avoidable on either theory. In the 1953 case it is avoidable only if the case is considered as a crossover. At present there is no method by which a lower limit can be set to recombination frequencies, nor an upper limit to " down " mutations. Their ranges may, in fact, overlap. Distinction must be made on other grounds than the theoretical frequencies of each. Nevertheless, it is usually safer to interpret a single rare event as a crossover rather than as a " down " mutation, since the general frequencies of the former have a much higher range than those of the latter. On the basis of the direction of mutation, then, the theory of two loci again has a slight advantage.

Finally, on evolutionary considerations, it appears that the theory of two loci has more probability than the other. It is difficult to understand why the two alternative genotypes on the theory of one locus, $Aa^t$ and $A^L$, should have, as a result of selection, the same phenotype : if the two phenotypes have been in competition, it is to be expected that the one which can become homozygous genotypically, and maintain the same phenotype, would have an advantage over the one which can only produce two homozygotes different in phenotype from itself and rather strongly different from each other. Selection does not usually favour quite such a large degree of adaptability to new surroundings as is thus shown by the genotype $Aa^t$, and in fact there is no certain case of polymorphism of *AA*, $Aa^t$, $a^ta^t$ known in the wild. In the knowledge that there are now environments which favour light-belly as well as dark, but that there are none favouring non-agouti dorsally, and assuming an almost equal mutation-rate for all members of the series, one would expect selection to result in almost equal dominance of *A* and $A^L$, and complete dominance of these two over $a^t$ and *a*. (Incidentally, if more were known about the factors modifying $A^L$ and *A* in the wild, it would probably be found that selection can produce interchangeable dominance between *A* and $A^L$.) On the theory of two loci with more or less independence of the direction of mutation at each locus, selection could work differentially on the back and the belly, and there would result the dominance interactions now observed : *A* is now dominant to *a*—in terms of this theory—and *W* to *w*, dominance of *W* being conditioned to some extent by the allele at the neighbouring locus * and modified by cumulative factors. And there

* The classification of the belly is easier in the presence of *a* than of *A*.

is no inconsistency in the phenotypic equivalence of the genotypes *AW/aw* and *aW/Aw*, these being the double heterozygotes in coupling and in repulsion respectively.

## 6. SUMMARY

1. A brief account is given of previous reports of mutations observed in the laboratory.

2. A new event at the agouti locus is described, which, unlike previous events, can be interpreted either on a mutational basis or as a crossover.

3. A brief account is then given of previous reports of light-belly observed in wild populations.

4. A discussion follows. An attempt is made to evaluate the evidence so far available on the number and kinds of activity which have occurred at the agouti locus.

5. From this it appears that there is much suggestive material, but not much real evidence, for the belief that $A^L$ is common in the wild and that consequently it can be regarded as a " wild-type " along with *A* with equal justification. More genetical tests must be made before such an assertion, which has much theoretical probability, can be made with any certainty.

6. From all the reports, a conservative estimate is made of the number of real mutations that have occurred. From this estimate, and from entirely theoretical considerations, arguments are put forward which indicate that the theory of two loci has slightly more in its favour than the single-locus theory. This appraisement is intended not so much in order to arrive at a final and certain conclusion—which is impossible on the present evidence—but in order to show what still needs to be done before a certain conclusion can be made.

I am indebted to Sir Ronald Fisher, F.R.S., for friendly discussion, which has stimulated my thoughts on this problem, and for criticism of the present paper.

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# A REVISED MAP OF THE FIFTH CHROMOSOME OF THE DOMESTIC RABBIT

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## 1. INTRODUCTION

CASTLE (1926) described a three-point backcross experiment in the rabbit involving the linked factors for Dutch spotting, English spotting, and Angora hair. He also considered that the three genes are located on the fifth chromosome of the rabbit in that order.

The Dutch pattern in rabbits is caused by a gene *p* which is almost completely recessive to self-colour *P*. The amount of white in this type of rabbit is also influenced by at least three minor recessive genes which hitherto are known to recombine freely with each other.

English spotting *En* is an incompletely dominant mutation which finds its expression in a complete inhibition of pigment formation in a considerable part of the coat. Its dominance is also influenced by the presence or absence of minor modifiers.

Angora hair *l* is the result of the increased length of the hair fibre found in certain breeds of rabbits. It behaves as a recessive to normal short hair.

The present work is undertaken as an attempt to present accurate recombination fractions between the three genes and to correct the hitherto known mapping of the fifth chromosome of the domestic rabbit in the light of a re-study and a re-calculation of the data presented by Castle (1924-1926).

## 2. DUTCH-ANGORA LINKAGE

In undertaking a comprehensive study of linkage in rabbits, Castle found that the Dutch and Angora genes are linked together with a recombination fraction of 14·26 per cent. The evidence for this linkage is summarised in table 1.

TABLE 1

*Double backcross data for (Dutch-Angora) linkage. Data from Castle*

| Nature of cross | ++ | *p*+ | +*l* | *pl* | Total |
|---|---|---|---|---|---|
| Coupling . . . | 97 | 20 | 13 | 72 | 202 |
| Repulsion . . . | 88 | 433 | 438 | 52 | 1011 |
| | | | | | 1213 |

Since the difference between the linkage values of the two sexes is not significant, all the data were combined together with the tri-coupling backcross data summarised in table 5.

TABLE 2

*Combined data for (Dutch-Angora) linkage*

| Nature of cross | ++ | *p*+ | +*l* | *pl* | Total |
|---|---|---|---|---|---|
| Coupling . . . | 330 | 54 | 42 | 313 | 739 |
| Repulsion . . . | 88 | 433 | 436 | 52 | 1011 |
| | | | | | 1750 |

Now we have a grand total of 1750 young of which 236 are cross-overs. This gives a recombination fraction of 13·48571 per cent.

## 3. ENGLISH-ANGORA LINKAGE

Castle also showed that the genes for English spotting and Angora are linked with a recombination fraction of 13·59 per cent. The evidence for this linkage is summarised in table 3.

TABLE 3

*Double backcross data for (English-Angora) linkage. Data from Castle*

| Nature of cross | *En*+ | *Enl* | ++ | +*l* | Total |
|---|---|---|---|---|---|
| Coupling . . . | 602 | 90 | 98 | 617 | 1407 |
| Repulsion . . . | 14 | 64 | 65 | 10 | 153 |
| | | | | | 1560 |

In connection with the original data of Castle, it should be noted that the matings designated as repulsion should be considered as coupling since all the heterozygous parents were of the constitution

TABLE 4

*Combined data for (English-Angora) linkage*

| Nature of cross | *En*+ | *Enl* | ++ | +*l* | Total |
|---|---|---|---|---|---|
| Coupling . . . | 835 | 120 | 132 | 857 | 1944 |
| Repulsion . . . | 14 | 64 | 65 | 10 | 153 |
| | | | | | 2097 |

*En*+/+*l*, and those designated as coupling should be considered as repulsion since the heterozygous parents were of the constitution *Enl*/++. Evidently the use of the terms coupling and repulsion

has been reversed. Coupling signifies that the two dominants are located in one chromosome and the two recessives in the other, irrespective of whether one or both mutants are dominant.

Here again the difference between the linkage values in the two sexes is not significant and all the data were, therefore, combined together with the tri-coupling backcross data summarised in table 5.

In a grand total of 2097 young there are 276 crossovers or 13·16166 per cent. recombination fraction.

## 4. ENGLISH-DUTCH LINKAGE

It appears now that the two spotting genes *p* and *En* are linked since both are linked with Angora with almost equal recombination fractions in each case. This indicates either that the genes for Dutch and English are close to the same locus or that they are on opposite sides of Angora and about equally distant from it. Castle determined

TABLE 5

*Tri-coupling backcross data for (English-Dutch-Angora) linkage. Data from Castle*

*En*++/+*pl*×+*pl*/+*pl*

| Short Dutch | Angora Dutch | Short English | Angora English | Angora Dutch English | Total |
|---|---|---|---|---|---|
| 34 | 240 | 233 | 29 | 1 | 537 |

the order by the introduction of Angora into a cross involving English and Dutch. Short-haired English rabbits have been crossed with triple recessives, *i.e.* long-haired Dutch, resulting in the production of triple heterozygotes *En*++/+*pl*. Tri-coupling backcrosses were then made and produced as a rule equal numbers of English and Dutch individuals. In addition, very rare crossovers may also be produced. These of course are of two kinds, *viz.* English-Dutch and self-coloured animals lacking both the Dutch and English factors. So far, Castle has obtained only one example of the first. Because of the rarity of crossovers Castle concluded that the two spotting genes are very closely linked, and located on the same side of Angora with a recombination fraction of 0·186 per cent. The evidence for this is summarised in table 5.

These data indicate that there is one crossover in a total number of 537 rabbits or 0·186 per cent. recombination fraction.

Castle says "Linkage tests English-Angora and Angora-Dutch, indicate that English is nearer to Angora than Dutch is, since the crossover percentage in the former case is 13·0±0·8 and in the latter 14·2±0·9. The data of table 17A (table 5 in this paper) indicate

the same relation. . . . This evidence is particularly convincing because it is based on matings in which both relations are simultaneously being tested ".

Now, examining the entire data of all the linkages we find that the data on Dutch-Angora give 236 recombinations out of 1750 or 13·48571 per cent. ; the data on English-Angora give 276 recombinations out of 2097 or 13·16166 per cent. So far as these data go they suggest that the Dutch gene is further from Angora than the English gene ; but this indication is very slight.

The $\chi^2$ test of significance in this case is that of the two by two table.

TABLE 6

*Two by two table*

| | Combinations | Recombinations | Totals |
|---|---|---|---|
| Dutch . . . | 1514 | 236 | 1750 |
| English . . . | 1821 | 276 | 2097 |
| Totals . . | 3335 | 512 | 3847 |

Using Yates' correction for continuity we have for $\chi_c^2$ a value of 0·06100698 only, whence $\chi_c$ is 0·2469959. The probability of observing a $\chi$ value as great or greater than 0·247, appropriate to the hypothesis that the locus for English is in the middle is 0·4025. On the opposite hypothesis that Dutch occupies the middle locus, we need the probability of observing a disproportion less than that observed or in the opposite direction. Consequently Yates' correction is reversed. $\chi_c$ now comes to 0·342315 and the probability to about 0·63394. The odds are, therefore, in the ratio 0·63394 to 0·4025 in favour of English lying in the middle.

But in the mating $En++/+pl \times +pl/+pl$ the only observed crossover between Dutch and English was Dutch-English-Angora, showing that the locus for English had in this case separated from the two loci for Dutch and Angora ; if English were in the middle locus, this would be a double crossover whereas if Dutch were in the middle locus it would be a single crossover only.

Now, if we accept Kosambi's formula for the relation between the recombination fraction and the map distance it appears that if $y_1$ and $y_2$ are the recombination fractions in the two segments then the frequency of recombination in both these segments is :

$$\frac{2y_1y_2(y_1+y_2)}{1+4y_1y_2}$$

so that of those gametes showing recombination in the second

segment the fraction which also show recombination in the first must be :

$$\frac{2y_1(y_1+y_2)}{1+4y_1y_2}$$

If now, as in our present problem, $y_2$ is exceedingly small, this fraction is reduced to $2y_1^2$.

Taking the two loci together there have been in all 512 recombinations with Angora out of 3847 or 13·30907 per cent. for $y$. Hence $2y_1^2$ is 0·035426 or 1/28·2, hence single crossovers are nearly 29·2 times as frequent as double crossovers among gametes showing the crossover between the English locus and the Dutch.

Combining the two probabilities we have calculated, we see that the combined event of a single crossover with a sampling deviation exceeding 0·247 of the standard error is about 18·3 times more probable than the combined event of a double crossover with a deviation exceeding −0·342.

In consequence of these calculations I am inclined to believe that the Dutch gene lies in the middle between the English gene and the Angora gene. The recombination fractions on this hypothesis will be as follows :

| | | |
|---|---|---|
| Angora-Dutch | . . | 13·216 per cent. |
| Dutch-English | . . | 0·186 „ |
| Angora-English | . . | 13·402 „ |

As we have determined the right order of the three genes for Angora, Dutch, and English, it remains to find the accurate recombination fractions between them.

Since such problems, in this as in many other cases, lead to troublesome algebraic expressions, it is useful to apply the arithmetical approach supplied by *Fisher's Scoring System* (1946). As in all iteration processes of approximation the precision required in working out the consequences of the trial values adopted is difficult to foresee, it is, therefore, usually convenient to carry out the work to the highest accuracy readily obtainable ; *e.g.* to use ten significant figures if a ten figure machine is employed. This course was followed in the work set out below, in which, however, the numerical values have been cut down before publication usually to seven significant figures, since it appears from the results arrived at that this number would have been sufficient.

Now, if we score $y_1$ and $y_2$ at the trial values 13·23 and 0·186 per cent. respectively we must therefore score $y_{12}$ at

$$\frac{y_1+y_2}{1+4y_1y_2} = 13{\cdot}40281 \text{ per cent.}$$

moreover

$$\frac{dy_{12}}{dy_1} = \frac{1-4y_2^2}{(1+4y_1y_2)^2} = 0{\cdot}9980205$$

$$\frac{dy_{12}}{dy_2} = \frac{1-4y_1^2}{(1+4y_1y_2)^2} = 0{\cdot}9281587$$

At 13·23 per cent. recombination fraction the score for $y_1$ (Angora-Dutch) will be :

| | | |
|---|---|---|
| $173/y$ | $173/0{\cdot}1323$ | $-1307{\cdot}634$ |
| $1040/(1-y)$ | $1040/0{\cdot}8677$ | $+1198{\cdot}571$ |
| | | $-\ 109{\cdot}063$ |

and the amount of information will be :

$$1213/(y)(1-y) \quad 1213/(0{\cdot}1323)(0{\cdot}8677) = 10566{\cdot}51.$$

At 13·40281 recombination fraction the score for $y_2$ (Angora-English) will be :

| | | |
|---|---|---|
| $212/y$ | $212/0{\cdot}1340281$ | $-1581{\cdot}75816$ |
| $1348/(1-y)$ | $1348/0{\cdot}8659719$ | $+1556{\cdot}63245$ |
| | | $-25{\cdot}12571$ |

and the amount of information will be :

$$1560/(y)(1-y) \quad 1560/(0{\cdot}1340281)(0{\cdot}8659719) = 13440{\cdot}80$$

Hence

| | Factor | Score | Information |
|---|---|---|---|
| For Angora-Dutch . . | 0·9980205 | −25·07597 | 13387·64 |
| For English-Dutch . . | 0·9281587 | −23·32065 | 11579·00 |

The geometric mean for the two values equals :

$$(13440{\cdot}80)(0{\cdot}9980205)(0{\cdot}9281587) = 12450{\cdot}50$$

In the case of the tri-coupling data, the expected frequencies of the four gametic types produced by the triple heterozygote could be expressed in terms of $y_1$ and $y_2$ only as represented in (table a). The total frequency is $1+4y_1y_2$ of which the two fractions, *i.e.* the original combinations and the recombinations, gives frequencies in those classes $(1-y_1)(1+4y_1y_2)$ gives old combinations for $y_1$ and $y_1(1+4y_1y_2)$ gives the recombinations for the same segment.

Similarly, fraction $1-y_2$ and $y_2$ of the total give frequencies for the segment $y_2$. Contingency table, simultaneous crossovers in both segments are represented by the following :

$$\tfrac{1}{2}\left(y_1+y_2-\frac{y_1+y_2}{1+4y_1y_2}\right) = 2y_1y_2(y_1+y_2)$$

since the frequency $2y_1y_2(y_1+y_2)$ is involved twice in a sum of total number of crossovers for both segments, *i.e.* $y_1+y_2$.

The other two tables (b and c) are constructed for $\frac{d}{dy}$ with respect to $y_1$ and $y_2$ respectively.

TABLE a

*Frequencies of the four gametic types produced by the triple heterozygote*

| | | $y_2$ | | |
|---|---|---|---|---|
| | | Old combinations | New combinations | |
| $y_1$ | Old combinations | $1-y_1-y_2+2y_1y_2(2-y_1-y_2)$ | $y_2-y_1y_2(y_1-y_2)$ | $(1-y_1)(1+4y_1y_2)$ |
| | New combinations | $y_1+2y_1y_2(y_1-y_2)$ | $2y_1y_2(y_1+y_2)$ | $y_1(1+4y_1y_2)$ |
| | | $(1-y_2)(1+4y_1y_2)$ | $y_2(1+y_1y_2)$ | $1+4y_1y_2$ |

TABLE b

*Differential coefficient with respect to $y_1$*

| | | |
|---|---|---|
| $1-4y_1+4y_1y_2+2y_2^2$ | $4y_1y_2-2y_2^2$ | $1+8y_1y_2-4y_2$ |
| $-1-4y_1y_2+2y_2^2$ | $-2y_2(2y_1+y_2)$ | $-1-8y_1y_2$ |
| $-4y_2+4y_2^2$ | $4y_2^2$ | $4y_2$ |

TABLE c

*Differential coefficient with respect to $y_2$*

| | | |
|---|---|---|
| $1-4y_1+4y_1y_2+2y_1^2$ | $-1-4y_1y_2+2y_1^2$ | $-4y_1+4y_1^2$ |
| $+4y_1y_2-2y_1^2$ | $-2y_1(2y_2+y_1)$ | $-4y_1^2$ |
| $1+8y_1y_2-4y_1$ | $-1-8y_1y_2$ | $4y_1$ |

Now considering that the values for $y_1$ and $y_2$ are 0·1323 and 0·00186 respectively, the three tables may be represented after substitution as follows :

TABLE a′

| | | |
|---|---|---|
| 0·8667583 | 0·0017958 | 0·8685541 |
| 0·1323642 | 0·0000660 | 0·1324302 |
| 0·9991225 | 0·0018618 | 1·0009843 |

TABLE b′

| | | |
|---|---|---|
| +0·9935512 | +0·0009774 | +0·9945286 |
| −1·0009774 | −0·0009912 | −1·0019686 |
| −0·0074262 | −0·0000138 | −0·0074400 |

TABLE c′

| | | |
|---|---|---|
| +0·5067909 | −0·9659777 | −0·4591868 |
| −0·0340223 | −0·0359909 | −0·0700132 |
| +0·4727686 | −1·0019686 | −0·5282000 |

The score equals $\frac{dm}{dy}/m$, but $m$ is equal to $a/A$ where $A$ is equal to $1+4y_1y_2$ and $a$ is equal to each fraction of the expectations. Therefore, $\log m = \log a - \log A$. Hence $\frac{dm}{dy} \times 1/m = 1/a \times da/dy - 1/A \times dA/dy$ and this will give the score.

Referring to the tri-coupling data illustrated in table 5, we find that the old combinations and the new combinations of the two segments, *i.e.* $y_1$ and $y_2$ are as follows :

| | | $y_2$ | | |
|---|---|---|---|---|
| | | Old combinations | New combinations | |
| $y_1$ | Old combinations . . | 473 | 1 | 474 |
| | New combinations . . | 63 | 0 | 63 |
| | | 536 | 1 | 537 |

The scores for $y_1$ are :

+1·153717 +0·5517025
−7·554865 −15·0107492

The scores for $y_2$ are :

+1·1133765 −537·38072
+0·2716441 −544·78798

The total score for $y_1 = +70·30335$.
The total score for $y_2 = +6·35994$.

The amount of information for the two segments is obtained in the form of a matrix consisting of $i_{11}$, $i_{12}$, $i_{22}$.

For $i_{11}$ the amount of information equals :

$$m(1/m \times dm/dy_1)^2 = 1/A\{1/a(da/dy_1)^2 - 1/A(dA/dy_1)^2\}.$$

For $i_{12}$ the amount of information equals :

$$m(1/m \times dm/dy_1)(1/m \times dm/dy_2) \ . \ . \ .$$

the product of the two informations

$$= 1/A\{1/a(da/dy_1)(da/dy_2) - 1/A(dA/dy_1)(dA/dy_2)\}.$$

For $i_{22}$ the amount of information equals :

$$m(1/m \times dm/dy_2)^2 = 1/A\{1/a(da/dy_2)^2 - 1/A(dA/dy_2)^2\}$$

| Information for $y_1$ | Information for $y_{12}$ | Information for $y_2$ |
|---|---|---|
| 1·138892 | 0·5809263 | 0·2963 |
| 7·756969 | 0·2572865 | 0·0874 |
| 0·000532 | −0·5257479 | 519·6076 |
| 0·014880 | 0·5403088 | 19·6182 |
| −0·000005 | −0·0039334 | −0·2798 |
| 8·911268 | 0·8488403 | 539·3297 |
| 4780·645 | 455·37900 | 289335·3 |

Now the whole data and calculations should be combined as follows for comparison :

| Test | Rabbits | Scores | | $i_{11}$ | $i_{12}$ | $i_{22}$ |
|---|---|---|---|---|---|---|
| | | A-D | D-E | | | |
| Angora-Dutch . | 1213 | −109·06300 | ... | 10566·51 | ... | ... |
| Angora-English. | 1560 | −25·07597 | −23·32065 | 13387·64 | 12450·50 | 11579·00 |
| Tri-coupling . | 735 | +70·30335 | +6·35664 | 4780·64 | 455·38 | 289335·30 |
| | | −63·83562 | −16·96401 | 28734·79 | 12905·88 | 300914·30 |

With these values we can write the equations :

$$28734{\cdot}79\, dy_1 + 12905{\cdot}88\, dy_2 = +63{\cdot}83562$$
$$12905{\cdot}88\, dy_1 + 300914{\cdot}30\, dy_2 = +16{\cdot}96401$$

where $dy_1$ and $dy_2$ stand for the adjustments required in the recombination fractions adopted. The solution of these equations is :

$$dy_1 = +0{\cdot}0022394 \quad +0{\cdot}22394 \text{ per cent.}$$
$$dy_2 = -0{\cdot}0000397 \quad -0{\cdot}00397 \text{ per cent.}$$

The accurate recombination fractions for $y_1$ and $y_2$ are 13·454 and 0·182 per cent. respectively. Using Kosambi's formula, the recombination fraction for $y_{12}$ will be 13·623 per cent.

The map lengths calculated from the recombination fractions obtained (using table VII, Fisher and Yates) are given in the summary.

## 5. SUMMARY

A correction of the hitherto known mapping of the fifth chromosome of the domestic rabbit has been made in the light of a re-study and a re-calculation of the data presented by Castle (1924-1926).

The new recombination fractions and the corresponding map distances between the three genes involved are as follows :—

| | Recombination fraction per cent. | Map distance |
|---|---|---|
| Angora-Dutch . . . . | 13·454 | 13·794 centimorgans |
| Dutch-English . . . . | 0·182 | 0·182 ,, |
| Angora-English . . . . | 13·623 | 13·976 ,, |

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# THE CHROMOSOME CONSTANCY OF THE NORMAL MAMMALIAN UTERUS

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## I. INTRODUCTION

MAMMALIAN chromosomes have mainly been examined either in cells of the testis in the male or in cells of the ovary in the female. These tissues, which contain the sex cells, have to be used for studies on meiosis so that they have also been used for studies on mitosis. In addition the testes contain the best normal cells for accurate chromosome counts so that most investigations have been restricted to this one tissue.

Other mammalian tissues have rarely been examined and there are only a few earlier studies on the chromosomes of normal somatic tissues. These have included reports on the occurrence of many somatic cells with less than the diploid number of chromosomes and they have always been a subject of controversy (Karplus, 1930; Kemp, 1930; Andres and Jiv, 1936). A more recent report on somatic tissues includes a study on the uterus of the human female. Here it is reported (Timonen, 1950; Timonen and Therman, 1950; Therman and Timonen, 1951) that the endometrium of the human uterus normally contains cells with numbers ranging from 4 to 104 chromosomes. The frequency distribution of these different chromosome numbers is reported as showing two main peaks. The highest peak is supposed to lie in cells with 20 to 25 chromosomes, with a much lower peak in cells with 45 to 50 chromosomes.

Some subdiploid variation can be found in certain mammalian tumours, and the comparison of normal and malignant tissues is an essential basis for an understanding of cancer. The existence of extensive subdiploid variation in normal somatic tissues would thus be of great importance in interpreting the origin of subdiploid cancer cells. A knowledge of the chromosomes of somatic tissues is also of importance for an understanding of the processes of development and differentiation.

The present study was therefore undertaken in order to see if extensive subdiploid variation is really a characteristic of normal somatic mammalian tissues. Many tissues of adult mammals have very few or no mitotic figures so that the chromosomes can no longer be studied after differentiation. But the endometrium is especially favourable in this respect as the uterine responses during the œstrus

cycle are correlated with periods of miotic activity. The most extensive subdiploid variation has been reported in the human endometrium and the present study therefore includes an examination of this human tissue. As the œstrus cycle in primates including man differs in some respects from that in other mammals, I have also examined the normal endometrium of the two rodents *Rattus norvegicus* and *Microtus agrestis*. This study on three different mammals provides concrete data on the chromosomes of normal somatic tissues.

## 2. MATERIAL AND TECHNIQUES

The human material used in the present study comes from women in England and in Israel. The endometrium was removed by biopsy and in all cases there were no signs of cancerous growth. The material from England was obtained through Dr J. O'Sullivan, Bethnal Green Hospital, London, and the material in Israel was obtained through Dr A. Mielnicky, Kupat Cholim, Jaffa. Immediately after removal from the body small pieces of endometrium were fixed in 1 : 3 acetic-alcohol or in 45 per cent. acetic acid and the material was then examined by the use of Feulgen squashes.

The rodents *Rattus norvegicus* and *Microtus agrestis* were reared in the laboratory and the animals were killed before the uterus was removed. Chromosome examinations were then also made by the use of Feulgen squashes (Sachs, 1952*b*, 1953*b*).

The chromosome picture which is obtained in a particular tissue depends not only on the mitotic activity and on the methods of fixation, staining and dehydration. It also depends upon the type of cell. Thus although the cornea of the eye and the epidermis of the ear in mammals show periods of high mitotic activity, these tissues are not the most suitable for accurate chromosome counts. The cells of the endometrium can give better chromosome pictures than either of these tissues, but even the endometrium does not give such good chromosome figures as those obtained from some spermatogonial cells. Counts of the exact number of chromosomes is therefore not always possible in the cells of the endometrium.

The chromosome counts in the present study have been made with an accuracy of about 5 per cent. of the chromosomes or even better. The application of more pressure in making the squashes might have produced an even higher degree of accuracy but this would have resulted in an excessive breaking of the cells which it is best to avoid. In the scoring of chromosome numbers I have taken great care that no damaged cells were included.

## 3. *HOMO SAPIENS*

A characteristic feature of the œstrus cycle in man and other primates is the existence of a period of uterine bleeding known as menstruation. Each cycle includes a period of growth and a period

of regression of the endometrium, and this is followed by bleeding and endometrial destruction during menstruation. After menstruation the endometrium is repaired during the growth period and the whole cycle is again repeated. The human cycle takes about 28 days and good mitotic activity in the endometrium is found during the growth period at about 7-12 days after the beginning of the cycle (Timonen, 1950). The biopsy material used in the present study was therefore taken at the growth stage of the human cycle.

The endometrial biopsies at this stage of the normal cycle were obtained from 2 women in England and from 1 woman in Israel. Chromosome counts were made on 50 cells and these have shown no variations from the normal ($2n = 48$) diploid chromosome number. It was also seen from the many dividing cells which were not so accurately counted that there is no extensive subdiploid variation in this somatic human tissue.

## 4. *RATTUS NORVEGICUS* AND *MICROTUS AGRESTIS*

The œstrus cycle in rodents is shorter than the cycle in man and there is no period of menstruation. The duration of the œstrus cycle in *Microtus agrestis* (the short-tailed bank vole) is not so exactly known. But *Rattus norvegicus* (the laboratory albino rat) has been extensively used for studies on the physiology of reproduction and it is known that the œstrus cycle takes about $4\frac{1}{2}$ days. In both species periods of mitotic activity have been found during the normal development of the endometrium.

The chromosome numbers in the testes are $2n = 42$ for *R. norvegicus* and $2n = 50$ for *M. agrestis*. The species *M. agrestis* is especially favourable for somatic chromosome studies since its two sex chromosomes are considerably larger than any of the autosomes and they can be readily identified during the stages of mitosis (Sachs, 1953*a*). Chromosome counts have been made on 50 endometrial cells in each of the two species and these have shown no variations from the normal diploid chromosome number.* It could also be seen from the many dividing cells which were not so accurately counted that there is no extensive subdiploid variation.

## 5. SUBDIPLOID VARIATION IN SOMATIC TISSUES

The present study on the chromosomes of the normal human endometrium, which is substantiated by the study on the two rodents, is thus in contradiction to the previous report on the human endometrium (Timonen, 1950). In both the present and Timonen's observations the biopsy material was taken at the same stage of the human cycle and the cytological study was based on the use of Feulgen squashes. There is no doubt that Feulgen squashes can give satisfactory

* The same situation was found in a count of 20 cells of the cornea of the albino rat.

results for mammalian chromosomes, but in the making of squash preparations one has to be very careful that the cells have not been damaged by the squashing (Sachs, 1953*c*). Excessive pressure will break the cells and this is especially true of prophase at which many of Timonen's counts were made. The many subdiploid and subhaploid chromosome numbers which have been reported in the human endometrium may have been due to the counting of broken cells. Another possibility is that previous observations have not been based on normal human material. The reported extensive subdiploid and subhaploid variation in other normal mammalian tissues (Therman and Timonen, 1951) should also be examined in the light of the chromosome condition in the normal endometrium.

There are some examples of normal mitoses in chromosome deficient pollen grains (Darlington and Mather, 1949). A certain amount of subdiploid variation has also been found in some plants (Sachs, 1952*a*), amphibians (Fankhauser, 1945), fish embryos (Svärdson, 1945), and mouse embryos (Beatty and Fischberg, 1951). But in these cases the cells with subdiploid chromosome numbers have been produced by environmental or by gene controlled mitotic abnormalities which are not characteristic of normal tissues. Subdiploid and subhaploid chromosome numbers have been reported in a " polyploid " rabbit (Melander, 1950) and a " polyploid " pig (Häggquist and Bane, 1951), but no such variation was found in polyploid implanted mouse embryos (Fischberg and Beatty, 1951).

Even in mammalian tumours, some of which show considerable variations in chromosome numbers (Koller, 1947), the existence of large numbers of subhaploid cells as a characteristic of malignant tumours is open to question. This is shown by studies on ascites tumours. These studies should be particularly accurate since ascites tumours are probably the easiest mammalian material for chromosome studies. These tumours consist of a suspension of free cells which are easily squashed and there is almost no chance of any appreciable errors in the counting of chromosomes. It is thus significant that studies on ascites tumours of rat (Makino, 1952) and of mouse (Levan and Hauschka, 1952) have not shown a single cell with a subhaploid chromosome number.

There is also some evidence on somatic chromosomes from cultures of mammalian tissues. But the conditions of a tissue in culture are very different from those existing inside the body and only slight variations in, *e.g.* the salt concentration of the medium, can alter the behaviour of the chromosomes and spindle. There is no doubt that good chromosomes figures can be obtained from mammalian tissue cultures (Hsu, 1952). But until the same tissues have been examined as they exist inside the animal one can never be certain how far any observed abnormalities are due to the conditions of the culture. Even with this possible drawback of the results from tissue cultures, chromosome counts on cultures of embryonic tissues of mouse, guinea-pig,

dog, and man, have all shown frequency peaks at the normal diploid chromosome number (Hsu, 1953).

It is not impossible that reduction, or increase, in chromosome number as a result of such mechanisms as chromosome lagging, non-disjunction, or an occasional multipolar spindle may normally occur in some somatic cells. But there is no evidence of anything like a higher frequency of subdiploid than of diploid cells, as the usual condition of normal somatic tissues.

## 6. POLYPLOID VARIATION IN SOMATIC TISSUES

The absence of extensive subdiploid variation in normal somatic tissues does not, however, mean that all somatic cells have the same chromosome number. In addition to an occasional polyploid cell in many tissues, and possibly some small percentage of subdiploid or hyperdiploid cells, a high frequency of polyploid cells has been found in the blood (La Cour, 1944) and liver (Biesele, 1944) of normal mammals, and in a variety of tissues in some other normal animals and plants (Huskins, 1947). Huskins assumes that genes must reproduce in order to survive and that the polyploidy is due to chromosome reproduction, which is essential for gene survival, in functional cells without adequate nuclear division.

There is, however, another explanation. It is of course clear that polyploidy as such is not necessarily a sign of degeneration and this can be seen from the existence of polyploid evolution. This is an adaptive form of polyploidy. But the high degrees of polyploidy in differentiated tissues, *i.e.* in the tissues which are not going to reproduce the organism, may merely be a sign that the cells are degenerating. This is then a pathological form of polyploidy. These cells may have become polyploid because they have lost some of their capacity for normal reproduction such as the ability to form functional spindles. In addition to such a loss as the ability to form spindles, further degeneration may result in a loss of the capacity for normal chromosome reproduction, thus leading to chromosome breakage. This condition has in fact been found in differentiated tissues of plants (Levan, 1951) which show many chromosome breaks in the highly polyploid cells. Similar chromosome degeneration may also occur in non-polyploid cells.

The same degeneration phenomena can be found in cancer tissues. It has often been assumed that chromosome abnormalities and great variations in chromosome number are a constant characteristic of cancers (Hauschka, 1952). But not all cancers have the same cytological picture, and during an investigation on a range of mouse tumours I have found some cancers which show no chromosome or spindle abnormalities. The behaviour of the different types of cancer can best be explained by the existence of basic tumour cells (Makino, 1952), these being the cells which keep the tumour going and which maintain the distinct pattern of each tumour. In the cancers with no abnormalities the basic tumour cells, and all the other cells of the

tumour, would have the diploid number of chromosomes. In other cancers the existence of these basic tumour cells with either the diploid, or in some types of cancer with the tetraploid chromosome number, can be inferred from studies on ascites tumours (Levan and Hauschka, 1952). The more than diploid cells in cancers with diploid basic tumour cells, and the more than tetraploid cells in cancers with tetraploid basic tumour cells, in addition to any cells with aneuploid numbers or chromosomes and spindle abnormalities in these cancers are thus merely the by-products of the basic tumour cells. The abnormalities observed in these cells are then the result of a loss of some of the normal reproductive capacity. In some cases basic tumour cells may have less than the full chromosome complement, so that some cancers may have near diploid or near tetraploid basic tumour cells (Makino, 1952 ; Hauschka and Levan, 1953).

This concept of basic tumour cells is also of importance in cancer chemotherapy. Decisions on the activity of chemical compounds on the basis of chromosome abnormalities produced in a particular tumour may merely be the judgment of compounds which kill already dying cells and leave the basic tumour cells untouched. The production of chromosome breaks in other organisms, such as the root tips of plants, whilst of great importance for an understanding of chromosome structure and behaviour, is therefore also of limited value in relation to tumour problems in mammals. In addition the absorption, utilisation, and effects of chemical substances in plants may be very different to the position which is found in mammals. This can be seen in the case of penicillin which is considered to be an effective mutagenic agent on the chromosomes of *Allium* root tips (Levan, 1951). Penicillin is, however, commonly used in man and the appropriate doses have shown no such drastic changes in human cells.

High frequencies of polyploidy and chromosome abnormalities can thus be found in some somatic tissues. But the existence of this chromosome duplication and of abnormalities may merely be an indication that the cells are no longer able to divide normally and that they have lost some of their reproductive capacity.

## 7. SUMMARY

The endometrium of the mammalian uterus is a favourable material for chromosome studies on normal somatic tissues. Uterine changes during the œstrus cycle are correlated with periods of mitotic activity in the endometrium so that mitoses can be examined even in adult animals. A study of the normal endometrium in *Homo sapiens* (the human female), *Rattus norvegicus* (the albino rat), and *Microtus agrestis* (the short-tailed bank vole), has shown no evidence of extensive subdiploid variation within this somatic tissue. There is also no indisputable evidence of extensive subdiploid variation as the usual constituent of other normal somatic tissues. Although some somatic

tissues contain high frequencies of polyploid cells, these cells and any with chromosome or spindle abnormalities may merely be degenerating cells which have lost some of their normal reproductive capacity.

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# THE GENETICS OF *BREVISTYLIS* IN *ŒNOTHERA* *

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## I. INTRODUCTION

THE mutant *brevistylis*, which differs from the normal in its short style and deformed stigma lobes, is one of the classic recessive mutations found by de Vries (1901-03) in *O. Lamarckiana* and shown by him to have a simple Mendelian inheritance. The gene is exceptional in showing segregation independently of either gametic complex in all *Œnothera* complex interchange heterozygotes in which it has been studied (Renner, 1942). This independence is shown particularly in a combination, such as *jugens. br gaudens* (Emerson and Sturtevant, 1932) which has a ring of 14 chromosomes at metaphase I of meiosis. Other combinations, in which *br* segregates independently of the complexes, are *albicans. velans*, *gaudens. flectens* and *rubens. flectens*, each with a ring of 14 chromosomes, and *albicans. gaudens* with a ring of 8 and a ring of 6 chromosomes (Renner, 1942). Hence, like the genes governing flower size, it must be at a locus genetically remote from those genes, or blocks of genes, which characterise the various complexes. Indeed there is evidence (Emerson and Sturtevant, 1932) of linkage of *brevistylis* to the flower size genes, with about 15 per cent. recombination. The demonstration of its position cytogenetically with considerable precision is therefore of more than trivial interest.

In the original stock, which was obtained from Prof. G. H. Shull in 1935, *O. Lamarckiana brevistylis* was, as de Vries originally found, completely female sterile owing to the extreme deformation of the stigma lobes, upon which pollen failed to germinate. The gene was transferred from this stock to the structurally homozygous *O. blandina*, by using pollen of *O. Lamarckiana brevistylis* on *O. blandina* stigmas. Amongst the progeny, which consisted of 52 *velans.* $^{h}$*blandina* and 3 *gaudens.* $^{h}$*blandina* plants, the *gaudens.* $^{h}$*blandina* combination heterozygous for the *br* gene was selfed. The *velans.* $^{h}$*blandina* combination is unsuitable to use as it is difficult to rid the progeny of heritable chloroplast defects. The progeny from the selfing of *br gaudens. br*$^{+}$ $^{h}$*blandina* consisted of 1 *blandina* plant that was *brevistylis* and 40 *blandina* and 4 $^{h}$*blandina. gaudens* plants that were not. Surprisingly the *blandina* plant homozygous for *br* was female fertile and all descendant stocks have also been female fertile.

The *blandina brevistylis* strain shows the short style effect very

* Dedicated to Otto Renner on his seventieth birthday.

clearly even in very young buds, the stigma lobes being at the mouth of the hypanthium or a little higher, but definitely below the level of the stamens. The stigmas in this strain are only slightly different from normal plants. It is possible that *O. Lamarckiana* contains genes which operate to make *brevistylis* more deleterious, but no direct test by returning the gene to *O. Lamarckiana* stock has been attempted.

Besides the stylar effects of the gene, which can be recognised in very young buds, the mature flower buds have a more stumpy appearance and the sepal tips are distinctly shorter and relatively thicker and are not appressed to one another as are those of normal buds. Further, the leaves of *brevistylis* plants are usually relatively broader and blunter, but this character is not a completely reliable one to employ in scoring families, where other characters are also segregating.

Identification of the chromosome bearing the *brevistylis* locus has been attempted by making trisomics heterozygous for *br* and searching for trisomic ratios in the backcross progeny. As the trisomics of *O. blandina* have not been described hitherto it is desirable to give first a brief account of them and of how their extra chromosomes have been identified.

## 2. THE TRISOMICS OF *O. BLANDINA*

### (*a*) *Primary*

The seven chromosomes of *O. blandina* have been identified, in terms of defined standard interchanges, as 1·2, 3·4, 5·6, 7·10, 8·13, 9·14 and 11·12 (Catcheside, 1940). All of the seven primary trisomics are known and each may be recognised phenotypically with very little trouble. Each is referred to by a distinctive nickname which recalls its most prominent diagnostic character. The descriptions indicate the features by which the trisomics differ from the diploid. Egg transmission is expressed as the percentage of the progeny of a trisomic, pollinated by a diploid, which are trisomic.

1. *Lanceolate*, trisomic for chromosome 1·2, is somewhat shorter, with more numerous, smooth, deeper green, lance-shaped leaves, generally more crowded on the stem than in the diploid. The buds appear polished and deeper red. The flowers are small, the anthers thin and the stigma lobes short, thick and often imperfectly divided from one another. Egg transmission 11 per cent.

2. *Whitish*, trisomic for chromosome 3·4, is a dwarf and weak plant with small elliptical leaves, which are rugose, white margined and flecked with numerous areas of tissue, white or yellow, that are defective in chlorophyll development. The bracts are short, relatively broad and patent. The buds are shorter and more slender and the sepal tips shorter and pink-tipped. Being so feeble, it breeds poorly and is difficult to maintain. Egg transmission 6 per cent.

3. *Broad*, trisomic for chromosome 5·6, has a strongly biennial tendency. The leaves are brighter green, very broad and blunt at the apex. They are strongly crinkled. The buds are stout, blunt and green. The petals are rather fleshy and often cleft at the sides. The stigma lobes are very stout. It is completely male sterile, the anthers being gnarled and indehiscent. Thus it is very similar in general

character to *O. Lamarckiana lata* in which chromosome 5·6 is also the extra one (Catcheside, 1937). Egg transmission 14 per cent.

4. *Linear*, trisomic for 7·10, has very long and extremely narrow rosette leaves, which are somewhat red margined. The leaves become shorter up the stem, passing into the linear, patent, nearly flat bracts. The buds are deeper red and are covered with a short pubescence. The flowers are smaller in all their parts. Egg transmission 12 per cent.

5. *Glossy*, trisomic for 8·13 or 9·14, is a slender, little branched plant, generally lacking basal branches. The leaves are short and somewhat wavy, and their upper surface has a high gloss at certain stages of growth. The stigma lobes are shorter and more slender and are borne at the level of the anthers. Egg transmission 28 per cent.

6. *Blunt*, trisomic for 9·14 or 8·13, has blunt spathulate leaves which are relatively broad for their length. The stigma lobes are relatively thick and large. Resembles " broad ", but is male fertile. Egg transmission 29 per cent.

7. *Golden*, trisomic for 11·12, has leaves which tend to be virescent, each leaf in development passing through a juvenile stage when it is golden yellow and then very slowly becoming green beginning from the apex and margins. The leaves are also relatively broader and shorter and strongly crinkled. The plants are slower in growing to maturity. Egg transmission 13 per cent.

The evidence identifying the extra chromosomes in the respective trisomics is as follows. The disomic gametes have been combined with various standard interchange complexes, and the trisomics examined cytologically. The complexes used were :—

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *ʰblandina* . . . | 1·2 | 3·4 | 5·6 | 7·10 | 8·13 | 9·14 | 11·12 |
| *blandina*-A . . . | 1·2 | 3·11 | 4·12 | 5·6 | 7·10 | 8·13 | 9·14 |
| *blandina*-B . . . | 1·4 | 2·3 | 5·6 | 7·10 | 8·13 | 9·14 | 11·12 |
| *rubricalyx*-α . . . | 1·2 | 3·4 | 5·6 | 7·14 | 8·13 | 9·10 | 11·12 |
| *ʰHookeri* . . . | 1·2 | 3·4 | 5·6 | 7·8 | 9·10 | 11·12 | 13·14 |
| *flavens* . . . . | 1·4 | 2·3 | 5·6 | 7·8 | 9·10 | 11·12 | 13·14 |
| *ʰpurpurata* (or *ʰfranciscana*, or " pale " *blandina*) | 1·2 | 3·4 | 5·6 | 7·10 | 8·9 | 11·12 | 13·14 |
| *velans* . . . . | 1·2 | 3·4 | 5·8 | 6·7 | 9·10 | 11·12 | 13·14 |
| *rubens* (or *gaudens*) . . | 1·2 | 3·11 | 4·9 | 5·6 | 7·12 | 8·14 | 10·13 |

The chromosome associations in the trisomics are written with the extra chromosome attached to the configuration containing the chromosome homologous to it. Thus 2+1 means it is a member of a trivalent, 4+1 that it is similar to one of the chromosomes in an interchange quadrivalent.

| | | |
|---|---|---|
| *Lanceolate—ʰblandina.* | *blandina*-B | 4+1, 2, 2, 2, 2, 2 |
| ,, | *blandina*-A | 2+1, 4, 2, 2, 2, 2 |
| ,, | *rubricalyx*-α | 2+1, 4, 2, 2, 2, 2 |
| ,, | *ʰHookeri* | 2+1, 6, 2, 2, 2 |
| ,, | *flavens* | 4+1, 6, 2, 2 |
| ,, | *ʰpurpurata* | 2+1, 4, 2, 2, 2, 2 |
| ,, | *velans* | 2+1, 8, 2, 2 |

The lanceolate chromosome is one of the two by which *ʰblandina* differs from *blandina-B*, namely 1·2 or 3·4. It is not one of the chromosomes, 3·4 and 11·12, by which *ʰblandina* differs from *blandina*-A, and so must be 1·2. The other observations agree with this.

*Whitish—blandina*-B. *velans* 4+1, 8, 2

The whitish chromosome is therefore 1·2 or 3·4, and so must be 3·4 since 1·2 is the extra chromosome in lanceolate.

| | | |
|---|---|---|
| *Broad*—*h blandina.* | *h Hookeri* | 2+1, 6, 2, 2, 2 |
| ,, | *flavens* | 2+1, 6, 4, 2 |
| ,, | *velans* | 8+1, 2, 2, 2 |

The broad chromosome is one of those which *h blandina* and *flavens* have in common, *i.e.* 5·6 and 11·12, and is one of those by which *h blandina* differs from *velans*, *i.e.* 5·6, 7·10, 8·13, 9·14. Only 5·6 is common to these possibilities.

| | | |
|---|---|---|
| *Linear*—*h blandina.* | *h purpurata* | 2+1, 4, 2, 2, 2, 2 |
| ,, | *velans* | 8+1, 2, 2, 2 |
| ,, | *rubens* | 10+1, 2, 2 |
| ,, | *h Hookeri* | 6+1, 2, 2, 2, 2 |
| ,, | *flavens* | 6+1, 4, 2, 2 |

The linear chromosome is not 8·13 or 9·14 from the combination with *h purpurata*, is not 1·2, 3·4 or 11·12 from the combination with *velans* and is not 1·2 or 5·6 from the combination with *rubens*. This leaves only 7·10, which satisfies all the observations.

| | | |
|---|---|---|
| *Glossy*—*h blandina.* | *h purpurata* | 4+1, 2, 2, 2, 2, 2 |
| ,, | *flavens* | 6+1, 4, 2, 2 |

The glossy chromosome is one of the two by which *h blandina* differs from *h purpurata*, namely 8·13 or 9·14.

| | | |
|---|---|---|
| *Blunt*—*h blandina.* | *blandina*-A | 2+1, 4, 2, 2, 2, 2 |
| ,, | " pale " *blandina* | 4+1, 2, 2, 2, 2, 2 |

The blunt chromosome is one of the two by which *h blandina* differs from " pale " *blandina*, namely 8·13 or 9·14. At present it is not possible to decide which should be assigned to blunt and which to glossy.

| | | |
|---|---|---|
| *Golden*—*h blandina.* | *blandina*-B | 2+1, 4, 2, 2, 2, 2 |
| ,, | *h franciscana* | 2+1, 4, 2, 2, 2, 2 |
| ,, | *blandina*-A | 4+1, 2, 2, 2, 2, 2 |

The golden chromosome is one of those by which *h blandina* differs from *blandina*-A, namely 3·4 or 11·12. It is one of those in common with *blandina*-B, and is therefore 11·12.

### (*b*) *Tertiary*

In addition to the primary trisomics, one tertiary (or interchange) trisomic, *subwhitish*, is known. It has been derived from the interchange *blandina*-A which possesses chromosomes 3·11 and 4·12 in place of 3·4 and 11·12 (fig. 1). The break in chromosome 3·4 is in arm 3, genetically just proximal to the P locus and cytologically in the heterochromatic proximal region of the chromosome arm. The break in chromosome 11·12 is in arm 12 in the distal euchromatic region. The result is that chromosome 4·12 is recognisably small

and unequal armed, the shorter arm being that carrying the translocated segment of arm 12. The argument leading to the identification of the smaller unequal armed chromosome as 4·12 is dealt with elsewhere (Catcheside, 1953).

Plants trisomic for 4·12 are shorter than diploids, with stiff stems, rather small broadly elliptical leaves which have a slight chlorophyll defect, similar to that of whitish, but obvious only in the seedling and rosette stages. The plants have many of the characters of whitish, but are nearly normally green and much more vigorous. Egg transmission 38 per cent.

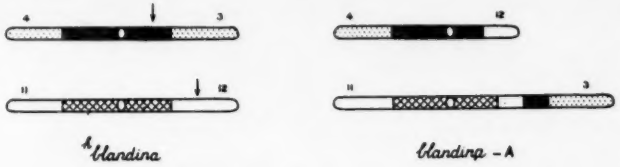



FIG. 1.—Diagrams of the chromosome structures differentiating *blandina* and *blandina-A* chromosomes. The black and cross-hatched regions near the centromeres are heterochromatic.

## 3. SEGREGATION OF *BREVISTYLIS* IN PRIMARY TRISOMICS

Only three of the seven primaries have been included in the test, since stocks of the remaining ones were not available at the time. In each case a normal primary trisomic was pollinated by a diploid *brevistylis*. The resulting trisomics, each heterozygous for *brevistylis*, were pollinated by diploid *brevistylis* and the resulting progenies classified with respect to the distribution of the gene amongst the diploids and the trisomics.

The trisomic whose extra chromosome carried the normal allele of *brevistylis* would on first crossing by *brevistylis* give trisomic progeny of constitution $++\ br$. Neglecting the effects of crossing over and possible double reduction, the $++\ br$ trisomics would give normal gametes in the ratio of $2 + : 1\ br$ and extra chromosome gametes in the ratio of $2 + br : 1 ++$. Hence the backcross $++\ br \times br\ br$ would show amongst the progeny a ratio of $2 +$ to $1\ br$ amongst the diploids and only $+$ amongst the trisomics.

Each of the remaining kinds of trisomics, whose extra chromosome does not carry the normal allele of *brevistylis*, would on first crossing by *brevistylis* give trisomic progeny of constitution $+\ br$. These on pollination by $br\ br$ would give equal numbers of $+$ and $br$ amongst the diploids and the trisomics. In considering the ratios, the diploid and trisomic progeny are taken separately, because the ratio of diploid to trisomic is affected by the reduced viability of gametes giving the latter.

Table 1 summarises the data obtained in this way. It is clear

that the *blunt* and *glossy* trisomics have shown disomic ratios for *br*, while the *golden* trisomic has shown trisomic ratios for *br*. It would be more satisfactory to have further tests to exclude the remaining chromosomes, nevertheless it is a valid conclusion that *br* is situated in chromosome 11·12.

TABLE 1

*Segregation of* brevistylis *in primary trisomics*

| | Progeny | | | | | | |
|---|---|---|---|---|---|---|---|
| | Diploids | | | | Trisomics | | |
| Heterozygous parent | + | *br* | $\chi^2$ for 1 : 1 | $\chi^2$ for 2 : 1 | + | *br* | $\chi^2$ for 1 : 1 |
| Diploids | 45 | 61 | ... | ... | ... | ... | ... |
| | 50 | 72 | ... | ... | ... | ... | ... |
| Totals | **95** | **133** | **6·33** | ... | ... | ... | ... |
| Blunt trisomic | **81** | **77** | **0·101** | ... | **44** | **41** | **0·106** |
| Glossy trisomics | 25 | 27 | ... | ... | 7 | 8 | ... |
| | 76 | 67 | ... | ... | 30 | 26 | ... |
| Totals | **101** | **94** | **0·251** | ... | **37** | **34** | **0·127** |
| Golden trisomics | 34 | 23 | ... | ... | 9 | 0 | ... |
| | 142 | 44 | ... | ... | 37 | 0 | ... |
| | 56 | 23 | ... | ... | 6 | 0 | ... |
| Totals | **232** | **90** | **68·8** | **4·2** | **52** | **0** | **52** |

The data are too limited to permit any conclusion as to the probable frequency of double reduction in trisomics. It could affect the frequency of the different genotypes only amongst the trisomic progeny. Most obviously, it would be shown by the appearance of *br br br* trisomics from the cross $++\ br \times br\ br$. It would also be shown by a shift in the ratio of $1 ++\ br$ to $2 +\ br\ br$ amongst the trisomic progeny such that $+\ br\ br$ would be relatively less frequent. Where random chromosome segregation predicts $1 ++\ br$ to $2 +\ br\ br$, random chromatid segregation predicts $6 ++\ br$ to $8 +\ br\ br$ to $1\ br\ br\ br$. Presumably, the actual ratios would lie between these limits.

One of the normal appearing trisomics in the progeny of the golden trisomic was pollinated by *br blandina*. The resulting family showed 5 normal and 16 *brevistylis* diploids, but no trisomics. Evidently the parent trisomic was $+\ br\ br$ and has given the $1 + : 2\ br$ ratio expected amongst its diploid progeny.

## 4. SEGREGATION OF *BREVISTYLIS* IN THE 4·12 TERTIARY TRISOMIC

The use of this trisomic allows the determination of whether or not *br* is in the segment of arm 12 present in the 4·12 chromosome. If it is not in the distal segment of arm 12, *subwhitish* trisomics heterozygous for *br* will show disomic segregation ratios for the gene amongst diploids and trisomics. If *br* is in the distal segment of arm 12, *subwhitish* trisomics will show trisomic segregation ratios, though these ratios will be different from those encountered in primary trisomics. In fact, the expected kinds of trisomic ratios were observed (table 2), showing that *br* is in the distal euchromatic region of chromosome arm 12.

TABLE 2

*Segregation of* brevistylis *in 4·12 tertiary trisomic*

| Constitution of parental chromosomes | | | Diploids | | Trisomics | |
|---|---|---|---|---|---|---|
| 11·12 | 11·12 | 4·12 | + | *br* | + | *br* |
| + | *br* | + | 36 | 32 | 10 | 0 |
| *br* | *br* | + | 17 | 78 | 32 | 2 |
| *br* | *br* | + | 10 | 85 | 29 | 8 |
| *br* | *br* | + | 6 | 74 | 29 | 9 |
| Totals . . . | | | 33 | 237 | 90 | 19 |

Diploid progeny obtained by pollinating a trisomic by a diploid must receive one of the two 11·12 chromosomes present in the trisomic parent. Trisomic progeny must receive the 4·12 chromosome together with one of the 11·12 chromosomes.

When a *subwhitish* trisomic, homozygous for $br^+$, is pollinated by a *br* diploid the trisomic progeny would have one 11·12 chromosome with *br* and the other with $br^+$, while the 4·12 chromosome would have $br^+$. Neglecting for the present the effects of crossing-over, such a duplex tertiary trisomic would produce *br* and $br^+$ haploid gametes in equal numbers, while its extra chromosome gametes would be equal numbers of 11·12 *br*+4·12 $br^+$ and 11·12 $br^+$+4·12 $br^+$. Hence pollinating a duplex tertiary trisomic with a *br* diploid should yield progeny in which the diploids show equal numbers of *br* and $br^+$ individuals, while the trisomics should all be $br^+$ in phenotype. The single progeny of this pedigree in table 2 shows this trisomic type of segregation, but the number of trisomic progeny is rather too small for complete assurance. On the assumption that the *br*

locus is in the 12 segment of chromosome 4·12, the trisomics in the above progeny should be of two kinds, one being 11·12 *br*+11·12 *br*$^+$+ 4·12 *br*$^+$ and the other 11·12 *br*+11·12 *br*+4·12 *br*$^+$. The former should behave like the parental duplex tertiary trisomic, while the latter, a simplex tertiary trisomic, should behave differently. Neglecting crossing over of the *br* genes, it would produce haploid gametes all 11·12 *br* and disomic gametes all 11·12 *br*+4·12 *br*$^+$. Hence, after pollinating by a *br* diploid, the progeny should show all diploids *br* and all trisomics *br*$^+$. Tests of three progeny trisomics of the duplex tertiary showed they were all of simplex constitution, since they gave ratios approximating to this expectation, but showing a small proportion (12·25 per cent.) of *br*$^+$ amongst the diploids and a similar small proportion (17·5 per cent.) of *br* amongst the trisomics.

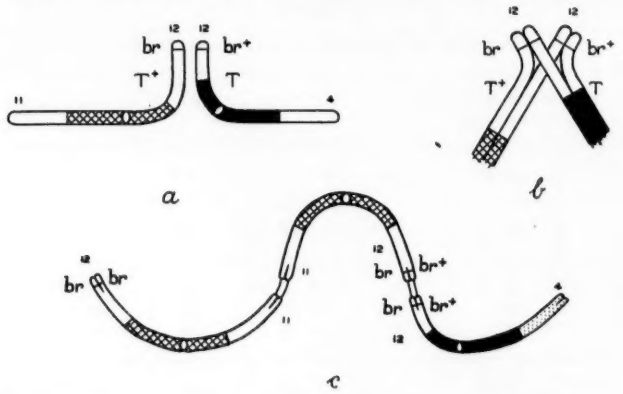



FIG. 2.—Pairing of a 4·12 chromosome with one of the 11·12 chromosomes in the tertiary trisomic, to show the origin of recombinations involving the *br* locus; (*a*) pachytene, (*b*) diplotene, (*c*) metaphase I.

The non-*brevistylis* diploid individuals represent cases in which *br*$^+$ has crossed over into the 11·12 chromosome, while the *brevistylis* individuals amongst the trisomics represent cases in which *br* has crossed over into chromosome 4·12. Crossovers between the two 11·12 chromosomes are not detectable. The two sorts of recombinant should be in equal proportion; a contingency test gives a $\chi^2$ of 1·87 for one degree of freedom, indicating agreement with the hypothesis of equality. The recombination value, for the interval between *br* and the interchange point in 4·12, calculated from the simplex data, is 13·72±1·77 per cent.

The section of chromosome concerned is that bounded distally by the locus of *br* and proximally by the place where the translocated fragment of arm 12 is attached to the stump of arm 3 (T of fig. 2). Presumably chromosome pairing at pachytene is two-by-two, the third chromosome in a trisomic being left out of partnership over a

given interval in a particular cell. Thus, ignoring changes of partnership within the T-*br* intercept, pairing of the terminal segment of 4·12 with its homologue in one of the 11·12 chromosomes (fig. 2*a*), followed by chiasma formation (fig. 2*b*) and chiasma terminalisation, would yield the chain trivalent shown in fig. 2*c*. The other, larger, segment of chromosome 4·12 would be capable of pairing with the homologous segment of either of the 3·4 chromosomes so that several different chromosome configurations would result at meiosis.

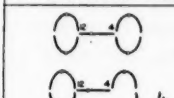
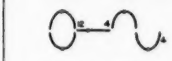
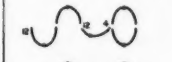
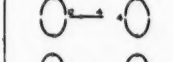
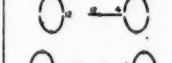
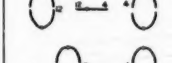
| TRISOMIC CONFIGURATION | REMAINING CONFIGURATION | | | | HALF XTA PER CHROMOSOME SEGMENT | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 12 | | | | 4 | | | |
| | | | | | 11·12 | | 4·12 | | 4·12 | | 3·4 | |
| | 5Ⓞ | 4Ⓞ + (Ⓞ) | 4Ⓞ + 2(1) | 3Ⓞ + 2(2) | MIN | MAX | MIN | MAX | MIN | MAX | MIN | MAX |
|  | 1 | | | | 1 | 15 | 1 | 2 | 1 | 2 | 1 | 15 |
|  | 1 | | | | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 15 |
|  | 4 | | | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 2 | 5 | | | 7 | 10·5 | 7 | 14 | 0 | 0 | 2 | 7 (5) |
|  | 5 | 6 | | 1 | 5 | 12 (7) | 0 | 0 | 12 | 24 | 12 | 18 |
|  | 3 | 8 | 1 | | 3 | 12 (9) | 0 | 0 | 0 | 0 | 3 | 12 (9) |
|  | 3 | 2 | | 1 | 6 | 6 | 6 | 6 | 0 | 0 | 3 | 6 (3) |
| | 11 | 5 | | | 11 | 16 (5) | 0 | 0 | 16 | 16 | 16 | 16 |
| | TOTAL CELLS 59 | | | TOTAL HALF XTA | 38 | 63 (21) | 19 | 27 | 34 | 48 | 42 | 64 (17) |
| | | | | MEANS | 0·64 | 1·07 | 0·32 | 0·46 | 0·58 | 0·81 | 0·71 | 1·08 |

FIG. 3.—Chromosome associations in the tertiary trisomic, their observed frequency, and the inferred half-chiasma frequencies in the trisomic segments.

The nature of these and their observed frequencies are shown in fig. 3. From these data it is possible to obtain approximate estimates of the frequency of half-chiasmata in segments 12 and 4. Uncertainties arise from two circumstances, one being that rod bivalents in certain cases may replace ring bivalents in the trisomic configuration ; the maximum possible number of these is shown in brackets in the figure. The other is that where a triple (terminal) chiasma occurs, the assignment of two of the half-chiasmata to the three possible chromosomes is arbitrary. The figures obtained therefore represent minimal and maximal estimates from the data. There is some indication of

preferential association of the 3·4 chromosomes with one another, but it is probably not very strong.

The frequency of chiasma formation between 4·12 and an 11·12 chromosome is between 0·32 and 0·46 per cell. The genetic length of the translocated 12 segment, measured cytologically as half the chiasma frequency, is therefore about 16 to 23 units. This is not much greater than the amount of recombination observed in the tertiary trisomic and indicates that the *br* locus is very close to the distal end of chromosome arm 12.

The recombination value measured above is not an ordinary one, since it represents only the frequency with which a gene at the *br* locus in chromosome 4·12 recombines with a gene at the *br* locus in either of the two 11·12 chromosomes with which it may associate in the tertiary trisomic. It does not measure recombination between the two 11·12 chromosomes, which must also occur within the same interval.

TABLE 3

*Expected segregations of* brevistylis *in 4·12 tertiary trisomics*

| Trisomic constitution | | | General formulæ | | | | Values for $a = 0{\cdot}14$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Diploids | | Trisomics | | Diploids | | Trisomics | |
| 11·12 | 11·12 | 4·12 | + | *br* | + | *br* | + | *br* | + | *br* |
| + | + | *br* | $1-a$ | $a$ | $\frac{4-a}{4}$ | $\frac{a}{4}$ | 0·86 | 0·14 | 0·965 | 0·035 |
| + | *br* | + | $\frac{1+a}{2}$ | $\frac{1-a}{2}$ | $\frac{2-a}{2}$ | $\frac{a}{2}$ | 0·57 | 0·43 | 0·93 | 0·07 |
| + | *br* | *br* | $\frac{1-a}{2}$ | $\frac{1+a}{2}$ | $\frac{a}{2}$ | $\frac{2-a}{2}$ | 0·43 | 0·57 | 0·07 | 0·93 |
| *br* | *br* | + | $a$ | $1-a$ | $1-a$ | $a$ | 0·14 | 0·86 | 0·86 | 0·14 |

If it may be assumed that the two 11·12 chromosomes do not pair with one another preferentially, but rather that, with respect to the T-*br* interval, they and chromosome 4·12 pair at random, the true recombination value would be half as great again as the apparent one, namely be 20·58 per cent. In so far as there was any preferential pairing of the two 11·12 chromosomes with one another, the true value would be even greater. No method is immediately available by which such preferential pairing and recombination could be measured.

There are four possible kinds of heterozygote in a tertiary trisomic and each yields a different ratio amongst its diploid and trisomic progeny, when pollinated by diploid *brevistylis*. Taking $a$ as the apparent recombination, namely the total recombination between the 4·12 and 11·12 chromosomes, the theoretical proportions of *brevistylis* given in table 3 may be calculated. The actual values, for the case $a = 0{\cdot}14$, are also given.

## 5. SEGREGATION OF *BREVISTYLIS* IN AN INTERCHANGE HETEROZYGOTE

The interchange heterozygote (figs. 1 and 4), from which the tertiary trisomic was obtained, provides an opportunity to see whether *brevistylis* shows pseudolinkage with genes on other chromosomes in the interchange. Only one segregating family, capable of showing this, has so far been examined. The heterozygote had $P^r\ br^+$ in the

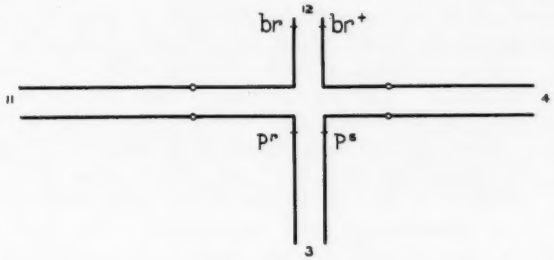



FIG. 4.—Diagram of pachytene pairing in interchange heterozygote, to show the relations of the *br* and *P* loci.

interchange (*blandina*-A) and $P^s\ br$ in the normal ($^h$*blandina*) chromosomes. It was pollinated by a homozygous $P^s\ br$ normal plant. The progeny are recorded in table 4. The means of classifying the interchange and normal plants are those previously described (Catcheside, 1947).

TABLE 4

*Progeny of* $\dfrac{A\ P^r\ +}{+\ P^s\ br} \times \dfrac{+\ P^s\ br}{+\ P^s\ br}$

| | $P^r$ | | $P^s$ | | Totals |
|---|---|---|---|---|---|
| | + | *br* | + | *br* | |
| *A* | 56 | 7 | 0 | 0 | 63 |
| + | 2 | 0 | 13 | 64 | 79 |
| Totals | 58 | 7 | 13 | 64 | 142 |

The non-recombinant classes are $A\ P^r\ +$ and $+\ P^s\ br$ and total 120. Recombinants in interval 1, between the *P* locus and the interchange locus, would be $A\ P^s\ +$ and $+\ P^r\ br$; none were present. Recombinants in interval 2, between the interchange locus and the *br* locus, are $A\ P^r\ br$ and $+\ P^s\ +$, totalling 20. Double recombinants in intervals 1 and 2 simultaneously, would be $+\ P^r\ +$ and $A\ P^s\ br$;

2 were found. Formally the recombination value in interval 1 is 1·4 per cent. (2/142) and in interval 2 is 14·8±3·04 per cent. (22/142). The data are peculiar in showing 2 double recombinants when there were no singles in interval 1. The conclusion they suggest, that the *P* locus is between the interchange and *br* loci, is not consistent with other far stronger evidence (Catcheside, 1953) which points to the relationship shown in fig. 3, nor with abundant previous work which has shown the *P* locus to be in chromosome arm 3.

The observed chiasma frequency between the 4·12 and 11·12 chromosomes in the interchange heterozygote is about 0·28 per cell, indicating the genetic length of the translocated 12 segment is about 14 units. This is consistent with the *br* locus being very close to the distal end of arm 12. The recombination value, for the *br* to interchange interval, in the interchange heterozygote, is not significantly different from the apparent value found in the tertiary trisomic. Nor is it different significantly from the " corrected " value in the tertiary trisomic, obtained on the assumption of no preferential pairing of the two 11·12 chromosomes with one another. The data are not consistent with the occurrence of any appreciable preferential pairing of this segment in the tertiary trisomic. Random pairing would be expected if zygotene association of the chromosomes began at the ends of the chromosomes, a conclusion suggested also by other lines of evidence, such as the frequent interlocking of bivalents at meiosis in *Œnothera*.

## 6. NON-DISJUNCTIONAL GAMETES AND THE *BREVISTYLIS* LOCUS

The interchange heterozygote, 3·4+4·12+12·11+11·3, produces predominantly the two kinds of disjunctional gamete 3·4+11·12 and 3·11+4·12. It also produces, very occasionally, two kinds of viable non-disjunctional gamete, 3·4+3·11 and 11·12+4·12, through adjacent chromosomes of the interchange ring passing to the same spindle pole at anaphase I of meiosis. The former has been called *A-Dp* and the latter *A-Df* (Catcheside, 1947), because the *P* and *s* loci are duplicated and missing respectively in the two kinds of gamete. This interpretation corrects a previous error in the understanding of *A-Dp* and *A-Df* and is fully discussed elsewhere (Catcheside, 1953). The gamete *A-Dp* lacks the distal segment of arm 12, while *A-Df* has it duplicated. If the placing of the *br* locus in the distal region of arm 12 is correct, a zygote composed of *A-Dp* and a normal gamete bearing the gene *br* should have the *brevistylis* phenotype, whereas a corresponding *A-Df* zygote should not. Several families containing plants of these constitutions have been grown and in each case the *A-Dp* and *A-Df* plants had the expected phenotypes with respect to *brevistylis*. The numbers of plants observed though not large, namely four of each type, are highly significant because the

cross in each case was of the type $\frac{A\ +}{+\ +} \times \frac{+\ br}{+\ br}$, the *brevistylis* gene coming only from the male parent.

## 7. SUMMARY

Primary trisomic segregation tests in *Œnothera blandina* show that the *brevistylis* locus is in chromosome 11·12. A tertiary (or interchange) trisomic segregation test places the locus very close to the distal end of arm 12. The constitutions of non-disjunctional gametes from an interchange heterozygote support this conclusion.

This is the first demonstration that a gene which shows free recombination with the differential segments in a complex heterozygote is located in the distal part of a chromosome. The location in the end of a chromosome, which is a pairing segment, with a probable mean of at least one chiasma in meiotic cells, accounts for the segregation being independent of that of the differential segments.

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# CHROMOSOME BEHAVIOUR IN SOME TROPICAL PLANTS

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## 1. INTRODUCTION

In this paper the chromosome numbers and other cytological features of certain tropical plants are reported. Some of the plants are of economic importance ; others, especially in the Commelinaceæ and Scitamineæ, are not. Frequent reference has been made to Darlington and Janaki Ammal (1945, abbreviated to D. & J., 1945) and to Tjio (1945). Sampson (1930) is the principal authority for nomenclature.

Root tips were taken from cuttings (RTC) or from seedlings (RTS) ; they were normally fixed in Craf or 2BE and stained in crystal violet but a few were successfully treated by the oxyquinoline-orcein method of Tjio and Levan (1950). For pollen mother cells, aceto-carmine following fixation in acetic alcohol was normally used. Propionic-carmine without prefixation was good in some cases.

## 2. THE OCCURRENCE OF TRIPLOIDY

Two of the plants examined are triploid ; significantly, both are vegetatively-propagated monocotyledons (like some hyacinths, tulips and bananas). One, *Xanthosoma helleborifolium*, is a tuberous weed and the other, *Costus sericeus*, a seed-sterile rhizomatous cultivated plant.

TABLE 1

*Meiosis in* Costus sericeus

| | | | | |
|---|---|---|---|---|
| Configurations | 9 II<br>9 I | 1 III<br>8 II<br>8 I | 8 II<br>11 I | 1 III<br>7 II<br>10 I |
| Cells | 13 | 3 | 1 | 1 |

The chromosome number of *Costus sericeus* was $2n = 3x = 27$. Pairing at meiosis was reduced (table 1). Anaphases were correspondingly irregular. This is suggestive of allotriploidy resultant from hybridity between distinct tetraploid and diploid species—AAAA × BB ⟶ AAB. Only a tetraploid has so far been reported (under *Costus speciosus*—Banerji, 1940 ; Raghavan and Venkatasubban, 1943). It

therefore seems likely that this extremely variable and confused species (or, better, species-complex) includes several distinct taxonomic entities and perhaps some hybrids between them. In Trinidad the plant is pollen- and seed-sterile but has considerable powers of spread and persistence by vegetative means; it has thus become well established in many of the damper parts of the island as an escape from cultivation.

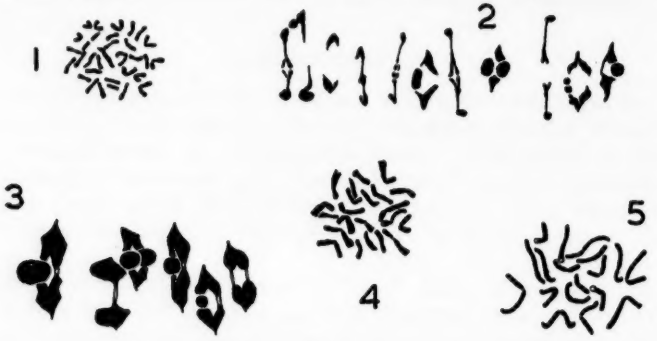


FIG. 1.

1. *Melicocca bijuga*. RTS. $2n = 32$. ×2100.
2. *Canavalia ensiformis*. PMC. $2n = 22$, 11 bivalents. ×2040.
3. *Sesbania speciosa*. PMC. $2n = 12$, 6 bivalents. ×2040.
4. *Tephrosia candida*. RTS. $2n = 22$. ×1860.
5. *Passiflora laurifolia*. RTC. $2n = 18$. ×2100.

## 3. THE OCCURRENCE OF INTERCHANGE

In *Strelitzia augusta* (Strelitziaceæ, $2n = 22$) a quadrivalent was present in 96 per cent. of the first metaphases examined (figs. 2-7). It

TABLE 2

*Meiosis in* Strelitzia augusta

| Quadrivalent | Present | | | | Absent |
|---|---|---|---|---|---|
| Configuration | Chain | | Ring | | |
| Orientation | Convergent | Parallel | Convergent | Not | |
| Cells | 5 | 2 | 1 | 16 | 1 |

was convergently oriented in only about 24 per cent. of cells and thus gave rise to considerable gametic sterility. Five out of 30 first anaphases showed numerical non-disjunction. Of a sample of 500 pollen grains, 34 per cent. were morphologically sound, in fair agreement

with the expected 26 per cent. calculated from first metaphase data (table 2).

The mean numbers of chiasmata per arm were 0·91 in both bivalents and quadrivalents. Thus, in *Strelitzia* with a relatively high chiasma frequency, association-in-four is virtually always achieved but orientation fails and sterility ensues. In *Musa acuminata*, by contrast, a related plant with chromosomes of similar size, chiasma frequency is lower (0·75 per arm, Dodds, 1943) and association-in-four is rare. Even though multivalents are usually convergent, sterility ensues though from quite a different cause (Dodds and Simmonds, 1948).

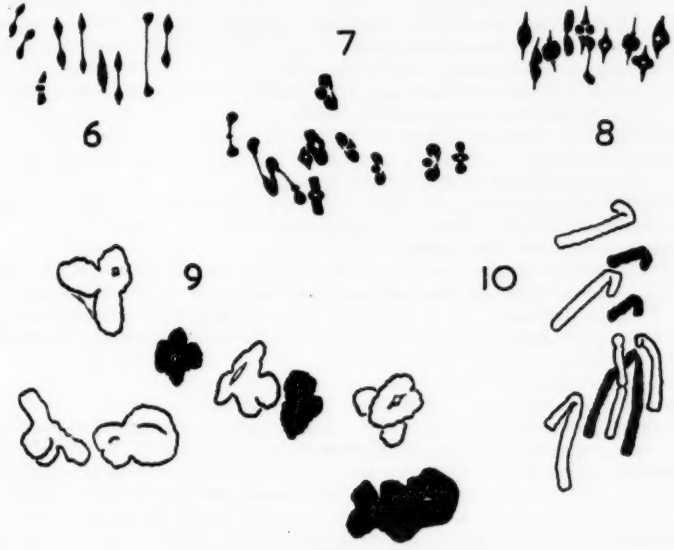


FIG. 2.

6. *Carica papaya*. PMC. $2n = 18$, 9 bivalents. ×2040.
7. *Strelitzia augusta*. PMC. $2n = 22$, 9 bivalents and one convergent chain quadrivalent. ×2040.
8. *Aneilema nudiflorum*. PMC. $2n = 20$, 10 bivalents. ×1620.
9. *Campelia zanonia*. PMC. $2n = 16$, 8 bivalents, the largest and two smaller pairs shown in black, the others in outline. ×1620.
10. *Campelia zanonia*. PMC. $2n = 16$, second anaphase to show chromosome shapes. ×1620.

In two members of the Commelinaceæ, the failure of occurrence of interchange is significant. Neither *Campelia zanonia* nor *Zebrina pendula* gave evidence of interchange hybridity though it had been recorded by previous investigators (Anderson and Sax, 1936; and Darlington, 1929, 1937 respectively). Evidently, structural hybridity is variable in these species, floating adaptively in the population as it does in *Campanula*.

## 4. LIST

New counts and counts differing from those prevously recorded are marked with asterisks

ANNONACEÆ. *Annona squamosa* L. Sugar apple. RTS. $2n = 14$.

*Annona muricata* L. Soursop. RTS. $2n = 14$.

BIXACEÆ. *Bixa orellana* * L. Anatto. RTS. $2n = 16$, differing from the $2n = 14$ of Janaki Ammal (D. & J., 1945).

TILIACEÆ. *Corchorus capsularis* L. Jute. RTS. $2n = 14$.

STERCULIACEÆ. *Theobroma cacao* L., *Th. bicolor* H.B.K., *Th. angustifolia* * Moc. & Sess. ex DC. *Herrania purpurea* * Pitt., *H. albiflora* * Goudot. Cacao, wild and cultivated. RTS, RTC. $2n = 20$ in all cases. The first two agree with Carletto (1946).

RUTACEÆ. *Citrus aurantiifolia* (Christm.) Swingle. Lime. PMC in aceto-carmine after acetic-alcohol fixation. $2n = 18$, 9 bivalents formed regularly.

*C. aurantium* L. Sour orange. RTS. $2n = 18$.

MELIACEÆ. *Cedrela odorata* * L. West Indian or cigar-box cedar. RTC. $2n = 50$-$52$, the chromosomes very small.

SAPINDACEÆ. *Melicocca bijuga* * L. Genip. RTS. $2n = 32$. (fig. 1-1).

ANACARDIACEÆ. *Anacardium occidentale* L. Cashew. RTS. $2n = ca$ 40 (*cf.* $2n = 42$ of Janaki Ammal in D. & J., 1945).

*Mangifera indica* L. Mango. RTS. $2n = ca$ 40, tentatively confirming Janaki Ammal (in D. & J., 1945) and Mukherjee (1950).

*Spondias mombin* * L. Hogplum. RTS. $2n = 32$.

LEGUMINOSÆ. *Canavalia ensiformis* DC. Jack, Sword or Horse bean. RTS. $2n = 22$. PMC in aceto-carmine after fixation in acetic alcohol showed regular formation of 11 bivalents whose structure could sometimes be resolved with remarkable clarity despite their comparatively small size (fig. 1-2).

*Canavalia plagiosperma* * Piper. RTS. $2n = 22$.

*Cyamopsis psoraloides* DC. Cluster bean. RTS. $2n = 14$.

*Erythrina poeppigiana* * (Walp.) O.F. Cook. Anauca immortelle. PMC in aceto-carmine. $2n = 42$, 21 bivalents regularly formed.

*Erythrina velutina* Willd. PMC in aceto-carmine. $2n = 42$, 21 bivalents regularly formed. Material taken from an ornamental tree introduced to Trinidad from Venezuela by A. H. G. Alston. Atchison (1947) also found $2n = 42$.

*Gliricidia sepium* * (Jacq.) Steud. Nicaraguan cocoa shade, Madre de Cacao. PMC in aceto-carmine. $2n = 22$, 11 bivalents regularly formed. This result differs from that of Atchison (1951) who found $2n = 20$.

*Indigofera endecaphylla* * Jacq. PMC in aceto-carmine. $2n = 32$, 16 small bivalents regularly formed.

*Phaseolus aureus* Roxb. Mung, green gram. RTS. $2n = 22$.

*Phaseolus mungo* L. Urd, wooly pyrol, black gram. RTS. $2n = 22$.

*Pithecellobium saman* Benth. Saman, Rain tree. RTS. $2n = 26$, as found by Atchison (1951).

*Pueraria hirsuta* * Scheid. (*P. thunbergiana* Benth.) Kudzu. PMC in aceto-carmine. $2n = 22$, meiosis normal.

*Saraca indica* L. PMC in aceto-carmine. $2n = 24$, meiosis normal.

*Sesbania speciosa* * Taubert. PMC in aceto-carmine. $2n = 12$, meiosis virtually normal with six large bivalents (fig. 1-3). Rare chromatid bridges without fragments were seen at first anaphase-telophase.

*Tephrosia candida* * DC. RTS. $2n = 22$ (fig. 1-4).

MYRTACEÆ. *Eugenia malaccensis* L. Pomerac. RTS. $2n = 22$.

*Eugenia uniflora* * L. Surinam cherry. RTS. $2n = 22$.

COMBRETACEÆ. *Terminalia catappa* * L. Almond. RTS. $2n = 24$.

PASSIFLORACEÆ. *Passiflora laurifolia* L. Belle apple, water lemon. RTC. $2n = 18$ (fig. 1-5) as found by Storey (1950).

CARICACEÆ. *Carica papaya* L. Papaw, papaya. PMC in aceto-carmine. $2n = 18$. Of first metaphases, 19 were regular with 9 small ring or chain bivalents (fig. 2-6); one contained 8 bivalents and 2 univalents. Of first anaphases, 29 were normal with 9-9 disjunction; one showed numerical non-disjunction; and another contained a bridge but no visible fragment.

RUBIACEÆ. *Coffea robusta* Linden, *C. excelsa* A. Chev., *C. liberica* Bull ex Hiern. Coffee. RTS. $2n = 22$ in each case.

BIGNONIACEÆ. *Bignonia unguis-cati* * L. Catsclaw. RTC. $2n = 40$.

*Callichlamys latifolia* * (L. C. Rich.) K. Schum. RTC. $2n = 40$.

*Crescentia cujete* * L. Calabash. RTS. $2n = 40$.

*Crescentia* (*Engallena*) *latifolia* * Mill. RTS. $2n = 40$.

*Jacaranda cærulea* * Juss. RTC. $2n = 36$.

*Kigelia pinnata* DC. Sausage tree. RTS. $2n = 40$.

*Paragonia pyramidata* * (Rich.) Bur. RTC. $2n = 40$.

*Phryganocydia corymbosa* * (Vent.) Bur. RTC. $2n = 40$.

*Pseudocalymma macrocarpum* * (Donn. Sm.) Sandw. RTC. $2n = 38$.

*Tabebuia pallida* * Miers. White cedar. RTS. $2n = 40$.

*Tabebuia serratifolia* * (Vahl) Nichols. Yellow poui. RTS. $2n = 40$.

*Parmentiera cereifera* * Seeman. Candle tree. RTS. $2n = 40$.

All species in this family except in *Crescentia* were counted by K. Shepherd.

NYCTAGINACEÆ. *Bougainvillea* spp. PMC in aceto-carmine. $2n = 34$ in several cases; irregularities of pairing and disjunction (bridge formation) indicated hybridity in some varieties, in accord with taxonomic expectation and in agreement with Wilson (1947). There is great need for a thorough cytotaxonomic study of the group which is technically quite favourable for cytological work.

POLYGONACEÆ. *Antigonon leptopus* * Hook. & Arn. Corallita. RTC. $2n = 42$-44, probably the latter, differing from 40 and 48 previously recorded (D. & J., 1945).

MYRISTICACEÆ. *Myristica fragrans* * Houtt. Nutmeg. RTS. $2n = 42$.

LAURACEÆ. *Persea americana* Mill. Avocado or Alligator pear. RTS. $2n = 24$.

PROTEACEÆ. *Grevillea robusta* A. Cunn. Silky oak. RTS. $2n = 20$.

EUPHORBIACEÆ. *Manihot utilissima* Pohl. Cassava, tapioca. RTC. $2n = 36$.

*Ricinus communis* L. Castor oil. RTS and PMC in aceto-carmine. $2n = 20$, meiosis regular.

ORCHIDACEÆ. *Vanilla planifolia* Andr. Vanilla. RTC. $2n = 32$.

STRELITZIACEÆ. *Heliconia psittacorum* L. Material from Piarco, Trinidad. PMC in aceto-carmine. $2n = 24$, meiosis virtually regular. 35 cells had 12 bivalents, two had 11 bivalents and 2 univalents. There was an average of 5·5 ring bivalents per nucleus and 6·5 rods.

*Heliconia bihai* (L.) L. Material from Arima-Blanchisseuse Road, Trinidad. $2n = 24$, meiosis regular. 13 cells had 12 bivalents each with an average of 7·8 rings per cell, perhaps a little higher than in *H. psittacorum*.

*Ravenala madagascariensis* Gmel. PMC in aceto-carmine. $2n = 22$, meiosis regular.

*Strelitzia augusta* Thunb. PMC in aceto-carmine. $2n = 22$. An interchange heterozygote (see section 3 above).

ZINGIBERACEÆ. *Costus cylindricus* (Plum.) Jacq. Material from the Arima-Blanchisseuse Rd., Trinidad. PMC in aceto-carmine. $2n = 18$, meiosis regular.

*Costus friederichsenii* * O. G. Peters. Material from the Arima-Blanchisseuse Rd., Trinidad. PMC in aceto-carmine. $2n = 18$, meiosis regular.

*Costus niveo-purpureus* * Jacq. Material from Diego Martin, Trinidad. PMC in aceto-carmine. $2n = 18$, meiosis regular, though rare failure of pairing in one bivalent was noticed.

ZINGIBERACEÆ—*continued.*

*Costus sericeus* * Blume (*C. speciosus* (Koenig) Sm. var. *argyrophyllus* Wall. ex Baker). Widely naturalized in Trinidad. PMC in aceto-carmine. $2n = 3x = 27$, a triploid (see Section 2, above).

CANNACEÆ. *Canna edulis* Ker-Gawl. Queensland arrowroot. RTC. $2n = 18$.

MARANTACEÆ. *Maranta arundinacea* L. West Indian arrowroot. RTC. $2n = 48$.

DIOSCOREACEÆ. *Dioscorea alata* * L. White Lisbon yam. RTC. $2n = 40$. Smith (1937) has recorded $2n = ca.$ 81.

COMMELINACEÆ. *Tradescantia elongata* * G. F. W. Mey. Material from the Arima-Blanchisseuse Rd., Trinidad. PMC in aceto-carmine. $2n = ca$ 50. Meiosis was extremely irregular with numerous laggards and bridges in both divisions; the plant was both male-and female-sterile.

*Tradescantia geniculata* * Jacq. Material from El Tucuche, Trinidad. PMC in aceto-carmine. $2n = 48$, meiosis regular, except for bridge and fragment at first anaphase (2/38 cells). Pollen was good and fruits were abundantly set. Anderson and Sax (1936) recorded $2n = 32$; thus the Trinidad plant is probably a hexaploid based on $x = 8$.

*Commelina diffusa* * Burm. fil. sensu Merrill. Material from the Botany Department, I.C.T.A., Trinidad. RTC. $2n = 30$. A new chromosome number for the genus *Commelina*.

*Commelina elegans* * H.B.K. Material from the Botany Department, I.C.T.A. RTC. $2n = ca$ 52.

*Aneilema nudiflorum* * (L.) Wall. Material from the I.C.T.A., Trinidad. PMC in aceto-carmine. $2n = 20$, meiosis regular (fig. 2-8). Pollen good and seeds freely set. The basic number of the genus is presumably 10, not 20 as indicated in D. & J. (1945).

*Campelia zanonia* H.B.K. Material from el Cerro del Aripo, Trinidad. PMC in aceto-carmine. $2n = 16$, meiosis regular (figs. 2-9 and 2-10), except for an occasional failure of pairing in a bivalent at metaphase. Pollen is abundant and seed freely set.

*Zebrina pendula* Schnitz. RTC and PMC in aceto-carmine. This plant is common in Trinidad (especially in cacao estates), but flowers have been seen only thrice and even then very few in number. Twelve bivalents were regularly found at first metaphase; about 1 per cent. of first anaphase-telophase stages showed a bridge with or without fragment.

PALMÆ. *Roystonea oleracea* * (Jacq.) O. F. Cook. Cabbage palm. RTS. $2n = 36$.

ARACEÆ. *Anthurium andræanum* Lind. Anthurium lily. RTC. $2n = 30$.

*Colocasia esculenta* Schott. Dasheen, eddoe, etc. RTC. $2n = 28$.

*Philodendron giganteum* * Schott. Material from Maracas Bay, Trinidad. RTS. $2n = 30$.

*Xanthosoma helleborifolium* * (Jacq.) Schott. Fowl foot bhaji (Trinidad). Material from the Botany Department, I.C.T.A., Trinidad, a weed. RTC. $2n = 39$. Presumably a triploid. It is propagated by its persistent tubers.

*Typhonium trilobatum* * (L.) Schott. RTC. $2n = 26$.

*Caladium bicolor* * (Ait.) Vent. Material from the Botany Department, I.C.T.A., Trinidad, a weed. RTC. $2n = 30$. Seed sterile and propagated by its persistent tubers.

GRAMINEÆ. *Pennisetum purpureum* Schum. Elephant grass, etc. RTC. $2n = 28$, in three distinct clones.

*Tripsacum australe* Cutler and Anderson. PMC in aceto-carmine. $2n = 36$, 18 bivalents in each of 10 diakineses; but, rarely, univalents were seen in other cells and a bridge with (probable) fragment at first anaphase. Pollen was abundant and seed was regularly set. The present count confirms that of Graner and Addison (1944).

GRAMINEÆ—*continued.*

*Tripsacum latifolium* Hitchc. Wild on hillside, Tunapuna, Trinidad. PMC in aceto-carmine. $2n = 72$, meiosis rather irregular with about 6 univalents per cell at diakinesis and 4·5 lagging at metaphase.

*Polytoca macrophylla* Benth. Material from Fiji collected by Dr K. S. Dodds and subsequently determined at Kew. PMC in aceto-carmine. $2n = 40$, meiosis regular.

## 5. SUMMARY

Chromosome numbers are reported for 87 species of tropical plants. There are included 38 new counts and 5 that differ from the published figures; information about meiosis is given for 28 species. Most of the plants studied are of economic importance. Some information is given on the occurrence of triploidy and interchange hybridity and upon the comparative cytology of the Scitamineæ and Commelinaceæ.

*Acknowledgments.*—I am indebted to Dr K. S. Dodds for help; to Mr N. Y. Sandwith for taxonomic determination of specimens of Scitamineæ and Commelinaceæ; and to Mr K. Shepherd for certain counts in Bignoniaceæ.

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# REVIEWS

**TREASURY OF HUMAN INHERITANCE, Vol. V, Part II. On syndactyly and its association with polydactyly. By Julia Bell. Cambridge University Press. 1953. 10s. 6d.**

This is the latest addition to the valuable collection of human pedigrees featuring pathological and abnormal conditions which during some forty years have been brought together in the *Treasury of Human Inheritance* under the sponsorship of the Galton Laboratory. From an exhaustive study of the genetical and medical literature, Dr Bell has assembled 63 pedigrees which include some 700 predominantly syndactylous digital anomalies. The varied manifestation of syndactyly, the association with polydactyly, differences in its sex incidence and the difficult problem of its inheritance, these are questions considered in a preliminary note. The genetical analysis of this material would certainly be of great interest. Dr Bell has classified the pedigrees on the basis of the varied manifestation and whilst this may be convenient, it can be only tentative for it is not demonstrated that these correspond with different genetical types. However, it does focus attention on interesting sex differences in the incidence of the various forms of this defect. Noteworthy amongst the collection is Schofield's unique pedigree (fig. 125) in which webbed toes are limited to males, being transmitted only from father to son, completely in accordance with the inheritance of a single gene on the Y-chromosome. One normal sister has been lost from this pedigree in transcription. J. H. BENNETT.

**NUCLEO-CYTOPLASMIC RELATIONS IN MICRO-ORGANISMS. Their bearing on cell heredity and differentiation. By Boris Ephrussi. Oxford : Geoffrey Cumberlege, Clarendon Press. 1953. Pp. 127. 18s.**

For forty years or so people have been studying the relations of nucleus and cytoplasm in animal and plant cells. The work has fallen into five main sections. First, there are the connections of nuclear and cytoplasmic heredity, chiefly in the higher plants. Secondly, there are the observations of bodies in the cytoplasm, primarily the chloroplasts of the higher plants, whose action in heredity could be traced. Thirdly, there are the relations of nucleus and cytoplasm under a variety of developmental and experimental conditions : they concern competition and co-operation of nuclei following abnormal chromosome and cell division induced by treatment, by cancer, by hybridity or by genotypic control. Fourthly, there are the chemical relations of nucleus and cytoplasm as determined both microscopically and also macroscopically in genetical experiments in a variety of organisms. Finally, it has become clear that the viruses of higher plants and animals—some multiplying in the cytoplasm, some in the nucleus, some spontaneous, some induced, and some natural, and all influenced by the character of the nucleus—all of them provide evidence on this problem.

During the last ten years most of these kinds of study have been transferred with prodigious success to micro-organisms. The more rapid and

sometimes more precise experiments and the different range of tests possible in this new field have vastly strengthened the conclusions reached earlier and have in some respects broken new ground. Moreover the future possibilities, as soon as microbiologists in general grasp the argument, are obviously enormous.

Professor Ephrussi, who has himself played an important part in this new development, here publishes three lectures which explain recent experiments with yeast, *Paramecium* and *Podospora*, all of them throwing light on these problems. He expresses his argument with the utmost lucidity so as to be intelligible to the general reader. Only in one sentence a phrase needs inserting (p. 81) " if crossing over occurs, there is post-reduction of mating type genes " would be better if it specificed " crossing over between gene and centromere ".

As a model of scientific exposition the three lectures reveal only one serious fault to the reviewer and that is one which most critics would take to be no fault at all. Ephrussi distinguishes (on p. 35) between what is " proved " or " demonstrated " and what is a " tempting assumption ". The same distinction arises later between what differentiation " is due to " and what it " may be due to " (p. 95). In the reviewer's opinion, however, scientific statements cannot be proved right. They can only be proved wrong ; and even that is more difficult than the world imagines. Is there not every gradation between what " is " and what " may be ", between the tempting assumption and the unquestioned truth ? At what point in the scale a particular statement stands we surely ought to judge only after the whole of the evidence has been considered. This is the rub. Do the higher plants and animals come in ? The multifarious evidence of the relations of nucleus and cytoplasm in them is not considered here. A paragraph explaining that it exists would strengthen the book. And if it were added that this evidence conforms in every imaginable way with that derived from micro-organisms nothing would have been lost. Indeed the analogy between *Paramecium* and *Pisum* or between " petite " yeast and the rogue tomato is the most far-reaching analogy to be found in genetics since it was discovered that crossing over explained linkage both in flies and in peas. Such analogies convert the tempting but gratuitous assumption into the well-founded hypothesis.

In a *Discussion*, Professor Ephrussi offers a model of the organisation of the egg necessary to account for differentiation. This provides a useful basis for enquiry. But perhaps of more immediate interest is his somewhat condensed *Addendum*. Here he goes deeper into the question of the relative function or importance of nucleus and cytoplasm. He advances the genetic evidence for the view that the " basic structure of cytoplasmic proteins is a function of cytoplasmic [as opposed to nuclear] elements ". Some contrast of this kind obviously exists. Probably it is not unconnected with the contrast within the nucleus between the properties of heterochromatin and euchromatin. It also poses the question as to whether we can admit that the direction of the cell is in the hands of a body that can merely adjust or titivate the imposing molecules which carry on outside it from generation to generation in substantial chemical continuity. But have we not here one of those paradoxes which constantly astonish us as we pass from the chemical to the cellular and from the cellular to the organismal level ? In many species a mere deficiency is all that distinguishes the two sexes

at the chromosome level. The most trivial difference in the nucleus determines the most profound difference in the organism. But we cannot on this ground regard the nucleus as trivial.

Following the same theme Professor Ephrussi points out that the nucleus. offers more opportunities to the experimenter to be tested and examined. This has given the nucleus, as it were, an unfair advantage over the cytoplasm. But to nature also does not the nucleus offer more opportunities to test its capacity? Surely it is to these opportunities for selection and adaptation that the long-term predominance of the nucleus, *i.e.* in heredity and evolution, is due. In development, however, the nucleus interferes, as Ephrussi has explained, in sequences of reciprocal reactions which are bound to be small and delayed if they are not to be disastrous. Importance therefore depends on the time scale. The village priest or policeman may be more important from day to day. But over a period of years the Pope or the President makes his weight felt—and simply by saying yes or no.

Such are a few of the problems raised by this book. It is a most timely and effective little volume. Later we may hope that it will appear in an expanded form. C. D. D.

**CLINICAL GENETICS. By Arnold Sorsby. Butterworth & Co. 1953. Pp. 577. 90s.**

Genetical studies of man are now rapidly increasing in importance both for genetics and for medicine. The reactions of the two are reciprocal. This situation is recognised in the present work by its division into two parts. There is an introduction on the theoretical side by twelve authorities, European and American, on the genetic interpretation of man. This is followed by a systematic clinical classification of diseases in over thirty sections.

With so many authors, and of so many nations, co-ordination is difficult and synthesis perhaps impossible. The fundamental account of chemical genetics, largely in fungi, cannot be brought into relation with all its bearings in the other articles without an explanation which it indeed deserves. The account of cancer cytology is not mentioned in the later account of cancer genetics although the connection between the two likewise deserves explanation, being more fundamental than either.

These gaps between different branches of medical genetics are of long standing and have led to a theoretical backwardness which becomes more noticeable as the subject becomes more important. The universal assumption that one-egg twins are genetically identical is adopted as a matter of course in this book. But if twin studies are to be taken seriously the error is a serious one. The assumption made here (on p. 36) that twins derived from the separate fertilisation of the opposite products of the second meiotic division in the egg would be more alike genetically than average sibs, has no serious consequences: it is merely of interest as showing another hiatus between medical genetics and genetics proper.

The deepest impression that one gets from this book is of the great importance that has been attached by both geneticists and clinicians to rare and striking abnormalities and the very slight interest they have taken in the genetics of susceptibility to infectious disease in general, and to the most widespread diseases, such as the common cold and dental decay, in particular.

The chapter dealing with these questions should be the most important in the book. But it is so condensed as almost to miss the point. The deep and general significance of Gowen's experiments is overlooked. And although the author seems to understand how natural selection operates on disease resistance, he does not seem at all anxious to admit the reader into his confidence on this illuminating method of approach.

The book as a whole, however, reveals the change that is taking place. The outstanding contributions from such varied fields as blood groups, psychiatry and ophthalmology show the new attitude towards medicine that genetics is introducing. The words " ætiology " and " congenital ", which are pre-genetic, are still used but they are not used here as a screen for ignorance. The authors realise that no study of disease which omits the genetic element can satisfactorily define the environmental element in causation. And this alone is a memorable contribution to medicine.

C. D. D.

# GENETICAL SOCIETY OF GREAT BRITAIN

## ABSTRACTS of Papers read at the HUNDRED AND THIRTEENTH MEETING of the Society, held on 7th NOVEMBER 1953, at the GALTON LABORATORY, University College, London, W.C. 1

### BIOMETRICAL ANALYSIS IN QUANTITATIVE INHERITANCE

B. WOOLF
*Institute of Animal Genetics, Edinburgh*

In his book *Biometrical Genetics* and several papers, Professor Mather has subjected the second degree statistics (variances and covariances) of quantitative characters in breeding experiments carried out over several generations to an arithmetical analysis which professes to provide estimates of the variance fractions ascribable to environment, the fixable and unfixable genetic variance, linkage, and gene interaction (departure from simple additivity), together with tests of significance. It will be shown that these computational methods can be simplified and made more direct. Each of the parameters to be estimated can be expressed as a linear function of the observations, the multipliers being usually quite small integers. Several different types of significance test are available and their applicability will be discussed. The question will then be raised how far and under what conditions the linear functions do in fact provide a valid measure of the genetic quantities with which they are labelled, and an indication will be given of some of the confirmatory information and tests that are desirable.

### THE ASSESSMENT OF DIFFERENCES IN MUTATION RATES PRODUCED BY TWO TREATMENTS TESTED IN A NUMBER OF EXPERIMENTS

F. YATES
*Rothamsted Experimental Station*

Experiments were carried out to test the existence of possible differences in frequency of lethals in the paternal X-chromosomes from irradiated spermatozoa of *Drosophila melanogaster* when the irradiation was given to spermatozoa which were harboured (*a*) in the females' receptacles and (*b*) in the males' testicles. It was desired to test the significance of the difference between the two methods of irradiation, allowing for the fact that in addition to changes in irradiation rate between the experiments there were liable to be other sources of variation in mutation rate from experiment to experiment. The test proposed was a combination of the significance levels of the separate experiments. The statistical problems arising in this test are discussed and an alternative approach based on estimation theory is given.

### SELECTION FOR LARGE AND SMALL SIZE IN MICE

D. S. FALCONER
*Institute of Animal Genetics, Edinburgh*

After 20 generations of selection with minimum inbreeding the rate of response remains undiminished in both large and small strains. Selection is three times more effective in reducing size than in increasing it. Several correlated characters showed correlated responses only during the first few generations.

## THE MEIOSIS OF A MALE SPECIES HYBRID BETWEEN *TRITURUS VULGARIS MERIDIONALIS* FEMALE × *TRITURUS HELVETICUS*

H. G. CALLAN
*Dept. of Natural History, University of St Andrews*

This hybrid newt shows the now familiar pattern of reduced chiasma frequency and heterozygosity for translocations. It is heterozygous for at least three independent reciprocal translocations. The parental species belong to a taxonomic group distinct from that including the various races of *Triturus cristatus* and *Triturus marmoratus*, in which translocations are already known to differentiate the races and species from one another. It thus appears probable that translocation heterozygosity is a systematic feature of newt hybrids.

## A SUGGESTION CONCERNING THE INHERITANCE OF "ACQUIRED CHARACTERISTICS"

J. L. CROSBY
*Dept. of Botany, Durham Colleges in the University of Durham*

The view is put forward that there might sometimes be a selective advantage in a mechanism which allows an adaptive response to unusual environmental conditions to be carried over in a hereditary manner to the succeeding generation. A possible mechanism, in accord with orthodox genetical knowledge and theory, is suggested ; it would, however, be limited to certain kinds of adaptive response.

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